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 shaping these networks. In a 13-year mesocosm experiment, we determined links between plant community stability and soil microbial networks. We found that plant communities on soil abandoned from agricultural practices 60 years prior to the experiment promoted destabilising properties and were associated with coupled prokaryote and fungal soil networks. This coupling was mediated by strong interactions of plants and microbiota with soil resource cycling. Conversely, plant communities on natural grassland soil exhibited a high stability, which was associated with decoupled prokaryote and fungal soil networks. This decoupling was mediated by a large variety of past plant community pathways shaping especially fungal networks. We conclude that plant community stability is associated with a decoupling of prokaryote and fungal soil networks and mediated by plant-soil interactions.

Introduction

Plants associate with complex interactive networks of soil microbial communities. These networks are increasingly found to act as a structuring force in plant community stability processes[1–5]. 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 in natural ecosystems[6,7]. 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[8,9]. To predict, protect and restore plant

communities, we need to understand the role of plant-soil-microbiota interactions in community stability processes.

Ecological interaction networks have a coherent structure with well-defined patterns in relation to network stability[8,9]. Network stability results from species connectiveness, negative interactions, few strong and many weak interactions and the clustering of species into subgroups[7,10–12]. 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[10,12–15].

Most of ecological network theory is derived from ecological food web theory, but has been shown to be applicable to microbial networks[7,10,13]. However, in comparison to food webs, microbial networks lack a strong directional structure and are based on co-occurrences of taxa alone[10]. Yet, we know that 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 by, for example, the input of dead organic material and root exudates as well as the uptake of soil nutrients[16,17]. The plant community may therefore play an essential role in shaping soil microbial network stability. We currently lack knowledge on the role of associations between the plant community and soil microbial networks in enhancing whole community stability.

Generally, interactions between plants and soil microbiota are increasingly found to act as a stabilising force in plant community processes[2–5]. Plant species identity and thus plant community composition is expected to determine the potential for stabilising mechanisms 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[2–5]. Direct interactions between plant community composition and microbial networks are therefore likely critical pathways in community stabilising mechanisms. Conversely, inherently more generic interactions of indirect plant community pathways mediated by soil chemical changes may result in microbiota responding in tandem. If such more generic effects are strong or not compensated for, these in tandem responses 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 interactions with soil microbial networks and test whether these plant community components associate directly or indirectly via soil chemical changes.

Here, we test to what extent and via which pathways overall and compositional plant community components associate with soil microbial biomass and networks. 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. 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[18]. Plant communities on natural grassland soils are typically stable communities, while communities on abandoned arable soil are more strongly impacted by invading plant species destabilising plant community networks[19,20]. We created diverse 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[18]. 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. Long-term plant community development resulted in communities with natural variation in, amongst others, plant invasion impact and plant community composition. We combined four datasets: plant community aboveground measurements over the 13 years and soil chemistry, total microbial biomass (PLFA/NLFA analysis) and soil microbial community composition (16S and ITS amplicon sequencing) after the 13th growing season.

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 direct and indirect pathways of the overall plant community (aboveground productivity and plant diversity) and plant composition onto 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 most important 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 associated with soil microbial networks and the role of plant-soil-microbial interactions in plant community stability.

Results

Natural grassland communities had a lower plant invasion and higher temporal stability

Plant community diversity gradually declined in time, but was drastically increased after the start of plant invasion on both natural grassland and abandoned arable soil (Fig 1A). Despite this variation in diversity, aboveground productivity of the communities remained relatively constant over time. In addition, aboveground productivity was, on average, 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, increased with the onset of invasion and was consistently higher in abandoned arable soil communities than in communities established on natural grassland soil (Fig 1C). For each community, we calculated stability over time and found that plant communities on natural grassland soil had a significantly higher temporal stability than plant communities on abandoned arable soil (Fig 1D).

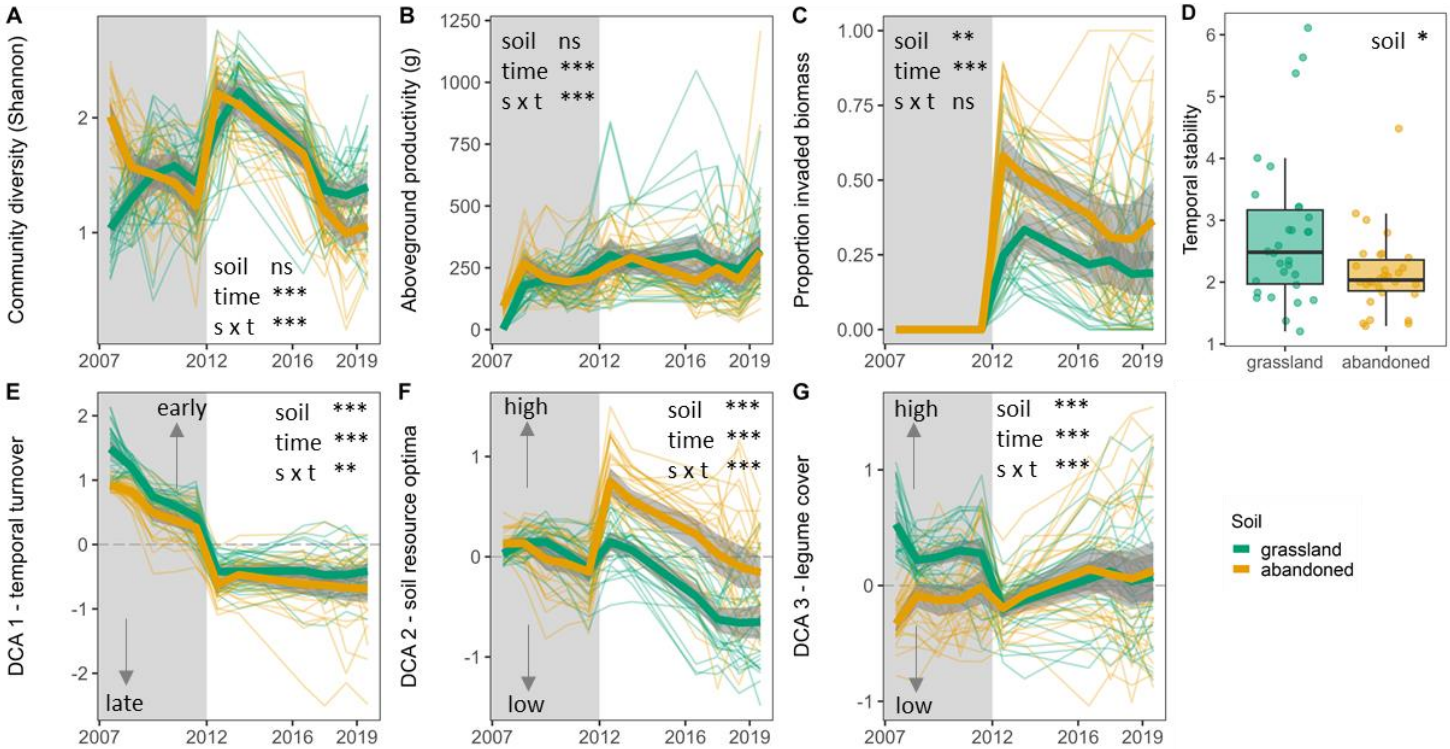

Figure 1 Plant community development over time on natural grassland (green) and abandoned arable soil (yellow). Plant community (A) diversity, (B) aboveground productivity, (C) biomass proportion of invaded plant species, (D) temporal stability based on aboveground productivity, and community compositional DCA scores related to plant species (E) temporal turnover, (F) ecological soil resource optimum and (G) legume cover. In E-G arrows with text indicate the interpretation of the DCA scores. 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. Thick lines indicate averages with the shading showing the 95% confidence intervals. Thin lines show each replicate (n = 30). Temporal stability was calculated as the inverse coefficient of variation of aboveground productivity over time. 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 the DCA axes, see Fig S1.

Plant community composition in time was analysed using detrended correspondence analysis (DCA). This analysis described variation in community composition by three axes: variation relating to

the gain and loss of species over time (temporal turnover separating early from late residency species; DCA 1), variation relating to Ellenberg indicator values of the plant species' soil resource optima (separating species with high and low soil resource optima; DCA 2), and variation relating to legume cover (separating communities with high and low legume cover; DCA 3) (Fig 1E-G, S1). DCA 1 showed a relative similar turnover in plant community composition over time between the two soil origins. In addition, on both soils a sharp drop in early residency species occurred with the start of plant invasion in 2012 (Fig 1E, S1A). For DCA 2, the start of plant invasion marked a strong increase in plant species with high soil resource optima as most invading species were characterised by having a high ecological soil resource optimum (Fig 1F, S1A). This increase was strongest for communities on abandoned arable soil and persisted in the following years (Fig 1F, S1A). Finally, DCA 3 separated plant community composition between the two soil origins before the start of invasion: communities on natural grassland soil harboured a higher cover of legumes than communities on abandoned arable soil in the years before 2012. With the start of invasion, plant community composition on natural grassland soil dropped to, on average, similar legume cover as for the abandoned arable communities (Fig 1G, S1B).

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 and microbial community composition (Fig 2AB, S2). 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 (Fig S2). Conversely, soil pH, fungal and AMF biomass, and belowground productivity were higher in natural grassland soil (Fig S2). Both prokaryote and fungal community composition were significantly different between natural grassland and

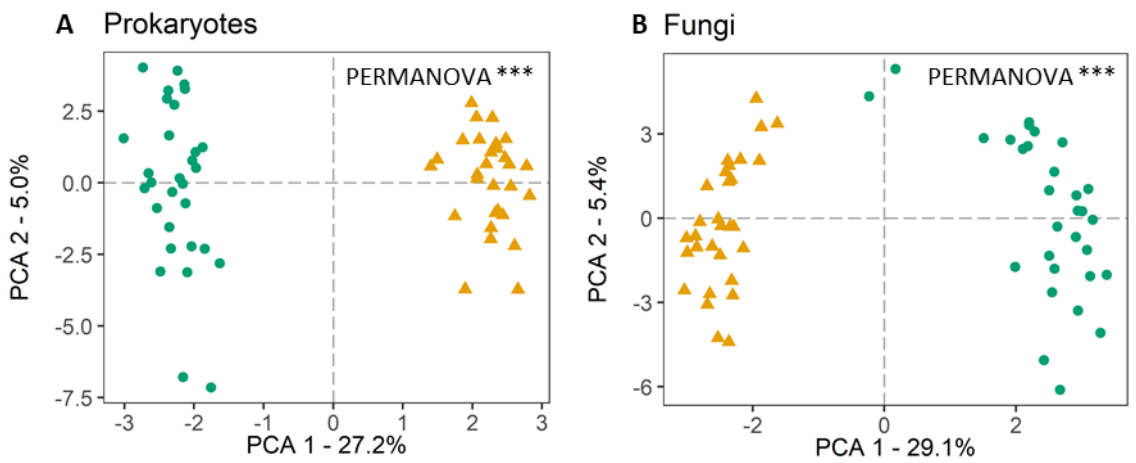

Figure 2 Principal Component Analysis (PCA) on clr-transformed (A) prokaryote 16S and (B) fungal ITS rRNA reads in plant communities established on natural grassland (green circles) and abandoned arable soil (yellow triangles). Amplicon sequencing was performed at the end of the 13th growing season (*n* = 30 per soil origin).

abandoned arable soil (Fig 2AB). These differences largely resulted from differences in prokaryote and fungal OTU abundances given the large overlap in the OTUs that were present in both soils (Fig S3AB).

Temporal stability was associated with a decoupling of prokaryote and fungal networks

For each soil origin, we created microbial co-occurrence networks. The networks indicated highly connected prokaryote and fungal communities in both natural and abandoned soil with few dominating OTUs and little difference in network properties commonly associated with network stability (Fig S4; Table S1). To understand the microbial network topologies in more detail, we clustered similarly responding OTUs across the 30 plant communities of each soil origin (Fig S4). Similarly responding prokaryote OTUs were captured in 9 and 10 clusters for natural grassland and abandoned arable soil, respectively. For fungi, 21 and 18 clusters were needed for natural grassland and abandoned arable soil communities, respectively (Fig 3AB). In all microbial networks, taxonomic families largely clustered together, indicating similar responses of closely related taxa (Fig S5-S6; Table S2-S5). Importantly, all microbial networks showed a significantly denser clustering of OTUs than

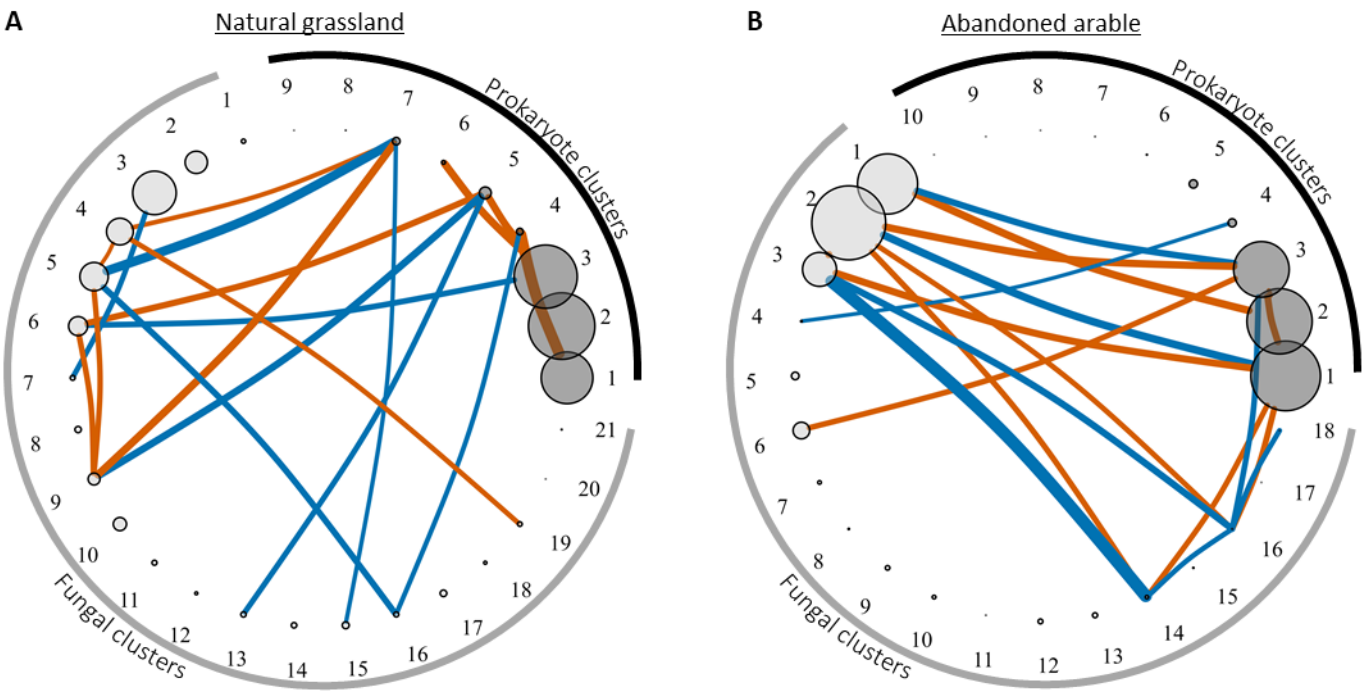

Figure 3 Correlations between prokaryote (dark grey) and fungal network clusters (light grey) in (A) natural grassland and (B) abandoned arable soil. Only significant correlations after Bonferroni correction for multiple testing are presented ($p < 0.0017$ and $p < 0.0018$ for natural grassland and abandoned 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 the prokaryote and fungal community each. Note that this means that cluster sizes can be compared between the two soils *within* the prokaryotes and fungal groups, but that prokaryote and fungal clusters are not scaled to each other and are therefore not directly comparable (but see Fig S2 for bacterial and fungal biomass comparison). For taxonomic and putative soil functions of each microbial network cluster see Fig S5-S6 and Table S2-S5.

147 would be expected based on null-models (randomly rewired networks; Fig S7), indicating a distinct
148 organisational pattern of each network.

149 We summed the relative abundance of the OTUs in each cluster. On average, three dominant
150 prokaryote clusters occurred that held, on average, 86% and 91% of the 16S rRNA reads recovered in
151 natural grassland and abandoned arable soil, respectively (Fig 3AB, S5AB). In abandoned arable soil,
152 these patterns were similar for the fungal networks showing 3 large clusters holding, on average, 75%
153 of the ITS rRNA reads. On natural grassland soil, however, fungal networks showed 5 larger clusters
154 holding, on average, 62% of the ITS rRNA reads (Fig 3AB, S5CD).

155 We tested for significant correlations between all clusters in each soil origin. Most strikingly, we
156 observed that in natural grassland soil, only one significant correlation between the dominant
157 prokaryote and fungal clusters was present, specifically between prokaryote cluster 3 and fungal
158 cluster 6 (Fig 3A). Conversely, in abandoned arable soil, several strong positive and negative
159 correlations occurred between the dominant prokaryote and fungal clusters, specifically between
160 prokaryote clusters 1-3 and fungal clusters 1-3 (Fig 3B).These findings suggest that responses of
161 dominant prokaryote and fungal clusters in natural grassland soil were decoupled, while in abandoned
162 arable soil, these clusters responded in tandem. Importantly, these patterns were not random and

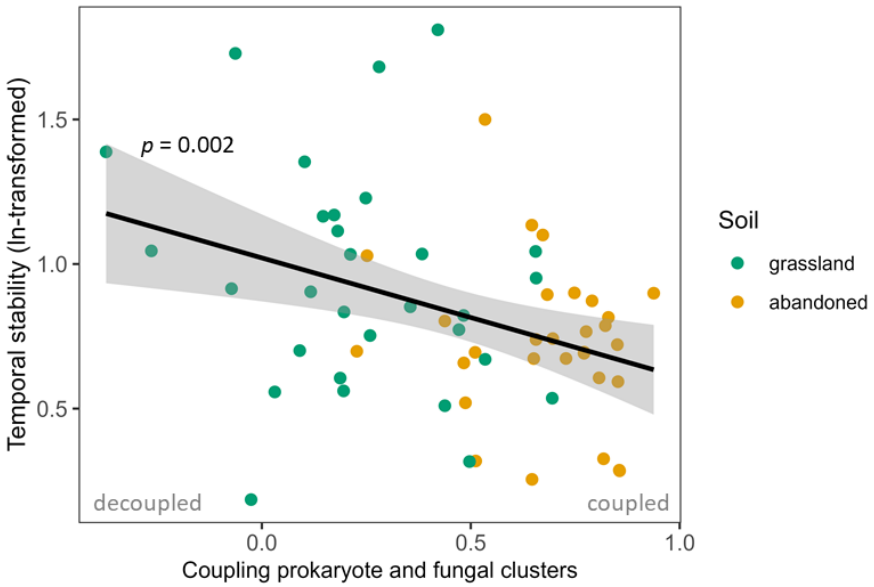

Figure 4 Correlation of temporal stability aboveground (ln-transformed) and the coupling of prokaryote and fungal clusters in soil microbial networks in natural grassland (green) and abandoned arable soil (yellow). Temporal stability was calculated as the inverse coefficient of variation in aboveground productivity of each plant community (Fig 1D). The coupling of prokaryote and fungal responses was estimated by the slope (beta) of the relative abundance of prokaryotes and fungi in network clusters 1 to 9 (Fig 3). The x-axis shows the beta slope coefficient. A positive value indicates that prokaryote and fungal reads were distributed similarly over the most prevalent network clusters, and thus, that prokaryote and fungal responses were coupled. A value of zero indicates that prokaryote and fungal read distribution over the network clusters was unrelated and thus decoupled. A few weak, negative correlations occurred and indicate that prokaryote and fungal read distribution over the network clusters were nearing an opposite pattern, indicating a strong decoupling of prokaryote and fungal responses. Significance from a linear mixed effects model including soil as a random factor is shown (n = 60). The black line indicates the average correlation with the shading showing the 95% confidence interval.

occurred significantly more often than expected by chance in both soil origins, highlighting the distinct organisational structure of the prokaryotes and fungi in the microbial networks (Fig S8).

For each plant community, we then calculated the strength of coupling between prokaryote and fungal network clusters. We found that the coupling of prokaryote and fungal clusters was significantly related to aboveground temporal stability: temporal stability was associated with a decoupling of prokaryote and fungal network clusters (Fig 4).

Microbial communities in abandoned arable soil were predominantly associated with the plant community in the year of sampling

We hypothesised that the plant community plays an important role in shaping soil microbial networks and biomass, and may underlie the coupling/decoupling of prokaryote and fungal networks. We tested this hypothesis using structural equation modelling (SEM). For each soil origin, we determined how plant community parameters from the year of sampling and from the past (initial plant invasion impact in 2012 and plant community developmental trajectories) associated with microbial biomass and network clusters at the end of the 13th growing season (Fig 5A). We tested whether these associations resulted from overall plant community factors (aboveground productivity and plant diversity) or plant community composition (DCA scores), and determined whether these pathways occurred via direct or indirectly pathways via soil chemical changes (Fig 5A). The obtained SEM models explained, on average, 47% of the variation in microbial biomass. For microbial clusters, the explained variation varied strongly between the network clusters in both soil origins and lay between 0 and 64% (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. Most strikingly, we found that plant community parameters in the year of sampling played a much larger role in shaping both microbial biomass and networks in abandoned arable than natural grassland soil (Fig 5B).

Furthermore, the main players in the year of sampling in abandoned arable soil were direct associations with the overall plant community and indirect associations of plant composition. In contrast, in natural grassland soil, associations in the year of sampling most consistently occurred via direct plant compositional pathways. Additionally, a few indirect plant compositional pathways occurred for prokaryote and fungal network clusters, but not for microbial biomass. Direct associations with the overall plant community occurred solely for fungal clusters (Fig 5B).

A variety of past plant community parameters were important in shaping microbial communities in natural grassland soil. Microbial biomass was largely shaped by direct and indirect pathways of past overall plant community factors. Prokaryote and fungal clusters were largely shaped by indirect

197 pathways of the past overall plant community as well as past direct compositional pathways (Fig 5B).
198 In abandoned arable soil, most of these past pathways were absent with exception of direct
199 compositional effects on prokaryote network clusters (Fig 5B).

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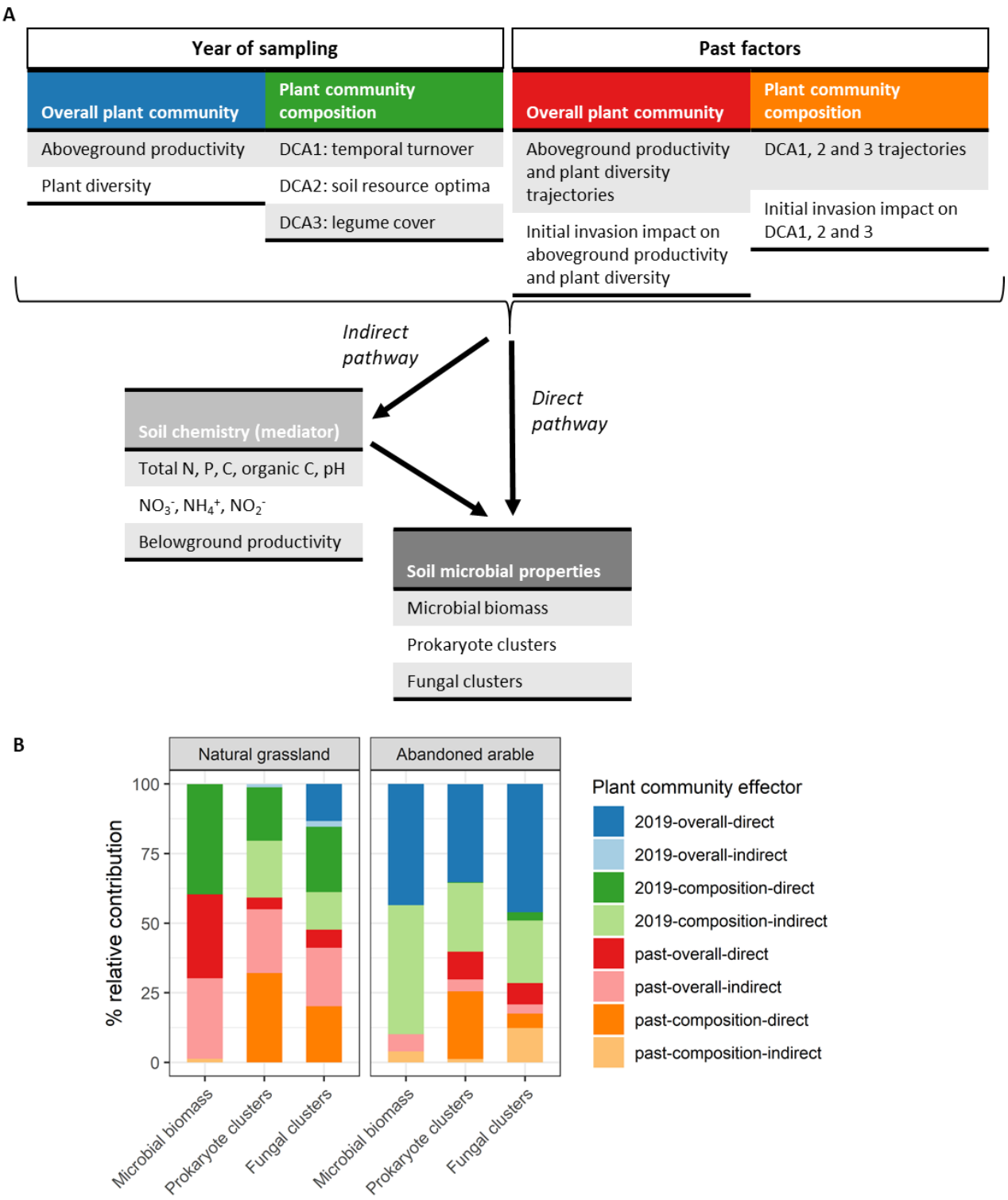

Figure 5 Structural equation modelling (SEM) coupling natural variation in plant communities, soil chemistry mediators (including plant belowground productivity effects), microbial biomass and microbial network clusters in plant communities established on natural grassland and abandoned arable soil. (A) Plant community factors implemented in the SEM approach from the year of sampling (2019) and the past (2012-2019), the considered soil chemical factors as mediators in indirect pathways and the soil microbial properties that were associated 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 weighted by the size of the involved microbial parameters (biomass or the average relative size of the network cluster). Values were 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 considered. In B, dark colours indicate direct pathways and faded colours indirect pathways. Note that the strict directionality of the indirect pathway is a mathematical necessity to test whether soil chemistry is a significant mediator, but should be interpreted as an interactions as both plants and microbiota influence soil chemistry. For information on the explicit pathways, see Fig S11-S12.

Prokaryote and fungal networks were associated with unique past pathways in natural grassland soil

To understand the mechanisms that underlie the association between stability and the coupling/decoupling of prokaryote and fungal soil networks, we compared the specific processes associated with prokaryote and fungal network clusters in natural grassland and abandoned arable soil. For this we extracted all direct and indirect plant community pathways with a relative contribution > 5% and determined whether these pathways occurred for both prokaryotes and fungal clusters or were unique to either group (Fig 6AB).

In natural grassland soil, both prokaryote and fungal clusters were for an important part shaped by unique pathways. These resulted especially from past plant community parameters (Fig 6A). For natural grassland prokaryote clusters, unique pathways resulted from plant compositional trajectories in time and, to a lesser extent, the initial effect size of plant invasion in 2012. Similarly, unique pathways for natural grassland fungal clusters resulted from plant compositional trajectories in time and the effect size of plant invasion, but different sets of plant compositional axes were involved. In addition, for fungal clusters, changes in soil pH and organic C played an important role in past pathways (Fig 6A).

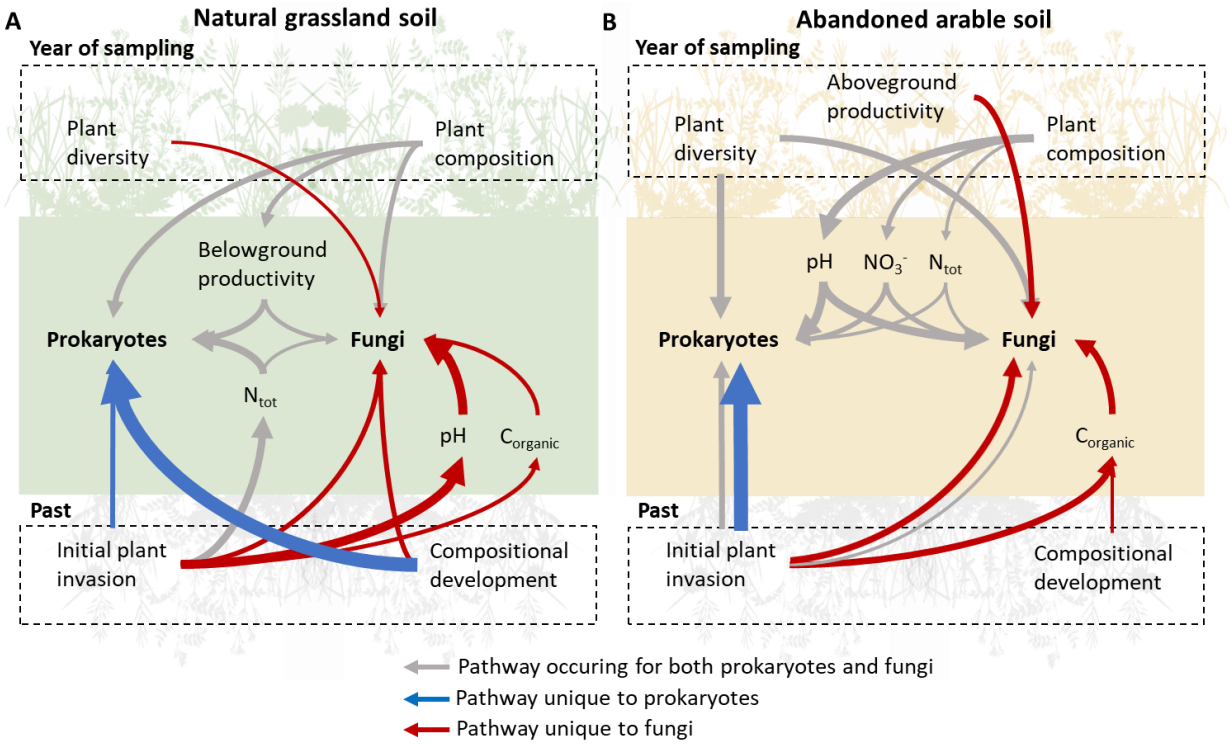

Figure 6 Summary of the most important pathways and plant community factors shaping prokaryote and fungal soil network clusters in (A) natural grassland soil and (B) abandoned arable soil. Grey arrows indicate pathways occurring for both prokaryote and fungal clusters, blue arrows pathways unique to prokaryote clusters and red arrows pathways unique to fungal clusters. Arrow width indicates the relative contribution of the pathway. Pathways related to plant community parameters in the year of sampling (2019; top part of figure) and from the past (2012-2019; bottom part of figures) were considered. The most important pathways were defined based on the relative contribution of plant community parameters (>5% contribution): the effect size of the pathway weighted by the relative cluster size (see Fig 3). Pathways are grouped and therefore multiple arrows can occur if, for example, different compositional axes are involved for prokaryotes and fungi.

In abandoned arable soil, less differentiation in past pathways between prokaryote and fungal networks occurred (Fig 6B). Some similarities to the natural grassland soil occurred, such as unique pathways for prokaryote and fungal clusters resulting from the initial effect size of invasion in 2012 (Fig 6B). However, on abandoned arable soil, overlapping pathways associating with plant diversity and composition in the year of sampling were more numerous than unique past pathways (Fig 5B).

In the year of sampling, plant composition decreased fungal community specialisation in abandoned arable soil

As a final step, we searched for generalities in the prokaryote and fungal characteristics that were associated with plant community pathways by testing which pathways significantly associated with overall microbial community patterns: alpha-diversity (Shannon's index) and the relative habitat specialisation index (SI). Additionally, we sought to understand the functional traits of each microbial network cluster by analysing their taxonomic composition and comparing it to existing literature and databases, and calculating the taxa's relative habitat specialisation and enrichment in natural grassland or abandoned arable soil (Fig S9-S10; Table S2-S5).

In the year of sampling, we found that plant composition in the year of sampling did not affect any of the soil nutrients in natural grassland soil, only belowground productivity was affected (Fig 6A; Table S7). As a result, most plant compositional pathways in the year of sampling occurred via direct interactions. A high legume cover in the year of sampling was associated with a low fungal and AMF biomass, and plant communities with a high cover of high resource optima plant species were associated with a low fungal diversity and bacterial biomass. In the latter case, belowground productivity was low and therewith increased prokaryote diversity (Table S7). Furthermore, in contrast to abandoned arable soil, plant diversity did not have detrimental effects on the fungal community and rather increased fungal diversity in natural grassland soil (Table S7). Most of the pathways in natural grassland soil involved metabolically diverse groups of bacteria, putative soil and litter saprotrophs as well as plant pathogens (Table S7).

In abandoned arable soil, we found that plant diversity and composition in the year of sampling associated with a large number of prokaryote and fungal network clusters. In contrast to natural grassland soil, the plant compositional pathways occurred via changes in soil pH, total N and NO_3^- in abandoned arable soil (Fig 6B). Most of these pathways involved dominant prokaryote and fungal clusters, putative soil saprotrophs, plant pathogens, ammonia oxidising archaea (AOA) and metabolically diverse bacteria (Table S8). Interestingly, a high cover of high resource optima plant species and a low cover of legumes resulted in a low fungal community specialisation, which was

mediated through an increase in soil pH (Table S8). Additionally, a high legume cover and high plant diversity decreased fungal biomass (Table S8; Fig S11B-S12).

Past plant community pathways were especially associated with putative saprotrophs and pathogens

In natural grassland soil, past plant community pathways were numerous and diverse, but for an important part related to the initial impact that plant species invasion had on the plant communities (Table S7; Fig S11). A large increase in aboveground productivity with the start of plant invasion in 2012 was associated with an increased soil N and decreased soil pH after the 13th growing season (2019). Subsequently, fungal and bacterial biomass as well as prokaryote community specialisation were increased. Similarly, a large increase in plant diversity with invasion in 2012 was associated with a decreased soil organic C (Table S7; Fig S12). These past plant community pathways involved a large diversity of microbiota, but especially diverse groups of chemoheterotrophic bacteria, putative soil saprotrophs and plant pathogens (Table S7).

Importantly, in natural grassland soil, large changes in plant composition with the start of plant invasion affected many fungal, but not prokaryote clusters. Prokaryote clusters were instead associated with plant compositional trajectories over time (Fig 6A; Table S7). The various fungal clusters affected by plant invasion impact especially consisted of putative soil saprotroph and plant pathogens (Table S7). For this latter group, plant communities where few early plant species were lost with the onset of plant invasion were increased in a specific set of putative soil pathogens. In addition, communities with a large increase in plant species with high soil resource optima were increased in a set of generalist soil pathogens, but decreased in another set of putative pathogens (Table S7).

In abandoned arable soil, past plant community pathways also occurred for an important part via the initial impact of plant species invasion in 2012. These pathways, however, consistently affected both prokaryote and fungal clusters and generally affected especially diverse groups of prokaryotes as well as putative soil saprotrophs and plant pathogens (Fig 6B; Table S8). Interestingly, almost all affected plant pathogens occurred in a single, dominant cluster, which was mediated by changes in soil organic C (Table S8).

Discussion

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

We tested how plant community stability associated with soil microbial networks. We found that above ground stability was associated to a decoupling of prokaryote and fungal responses below ground. Soil abandoned from agricultural practices 60 years before the start of the 13-year long mesocosm experiment had destabilising effects on dry grassland community plant-soil-microbial networks, compared to natural grassland soil. This instability was demonstrated by a lower aboveground temporal stability and a higher success of invading plants in communities grown on abandoned arable soil, which is in line with previous studies[19,20]. Furthermore, in abandoned arable soil, the dominant fungal and prokaryote network clusters had strong positive and negative co-occurrences, indicating a joint response to environmental factors. This response was tied to the plant community, as both the prokaryote and fungal clusters were associated with relatively similar pathways related to plant-soil interactions. These patterns are characteristic for instable networks, as perturbation effects can easily spread throughout the network, affecting large proportions of a network rather than just a small section (Fig 7)[11,21]. This highlights the sensitivity of the abandoned arable microbial networks to disturbances and the potential for major restructuring.

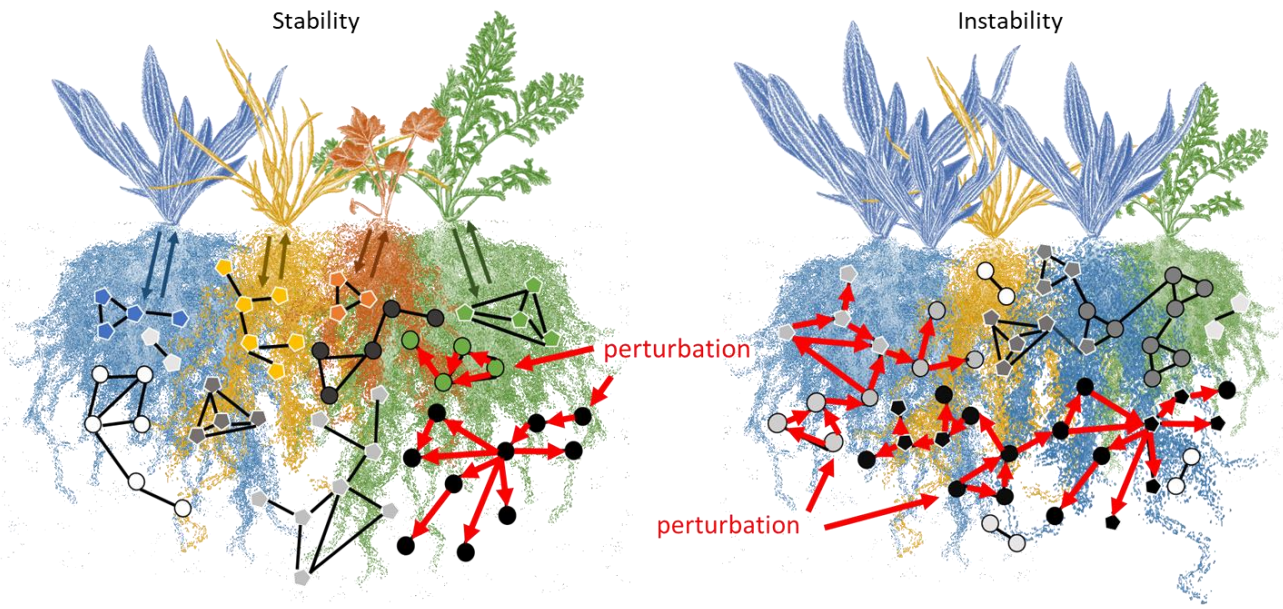

Figure 7 Conceptual framework reconciling the importance of the decoupling of prokaryote and fungal responses in buffering the propagation of local perturbation effects. In (A) stable settings, prokaryotes (circles, black border) and fungi (pentagons, white border) occur in separate clusters. This separation results from different factors shaping prokaryote and fungal niches. For example, fungal clusters are for an important part directly associated with different plant species (colours of fungal pentagons match colour of the plant species). The resulting microbial networks are likely to buffer local disturbance effects as effects on a subset of the occurring clusters are not likely to spread to unconnected clusters (red arrows showing the spread among connected taxa). In (B) unstable settings, prokaryotes and fungi respond in tandem and create three dominant clusters. This coupling results from a few dominant factors shaping both prokaryote and fungal soil network clusters. The resulting microbial networks are likely to spread disturbance effects throughout large parts of the network given the high connectiveness of dominant clusters (red arrows).

293 In contrast, we found that prokaryote and fungal responses in natural grassland soil were largely
294 decoupled. The prokaryote and fungal networks clusters were, for an important part, associated to
295 separated pathways related to the plant community. This division likely created separate soil niches
296 for each group[22,23], leading to enhanced resilience against disturbances in the system. The decoupled
297 structure of microbial networks is associated with the ability to buffer against the spread of
298 disturbances: a disturbance affecting one or a few of the microbial clusters will not cascade into
299 affecting unconnected clusters (Fig 7; compartmentalization[11,21]). We conclude that plant community
300 stability is associated with a decoupling of prokaryote and fungal responses, which is likely shaped by
301 interactions with the plant community.
302
303 *Plant-mediated soil legacies play a key role in decoupling microbial networks*
304 We show that the soil microbial networks in natural grassland soil were more strongly associated with
305 past plant community pathways than the networks in abandoned arable soil. These past plant
306 community pathways may have separated the responses of prokaryote and fungal network clusters in
307 natural grassland soil. This suggests that plant-mediated soil legacies play a crucial role in decoupling
308 fungal and prokaryote responses, resulting in a more stable soil microbial community (Fig 7).
309 Plant-mediated soil legacies are well-known phenomena and play a driving role in plant community
310 coexistence, diversity and succession[2–4]. We found that plant-mediated soil legacies resulted, for an
311 important part, from the initial impact of plant species invasion in 2012. In natural grassland soil,
312 drastic increases in plant diversity, aboveground productivity and changes in plant composition
313 resulted in numerous changes in putative fungal saprotrophs after the 13th growing season. Together
314 with the lasting impact of the initial plant invasion on soil organic C, pH, and total N, our findings
315 suggest that past invasion pathways were largely due to changes in decomposition processes. This is
316 consistent with the observed association of these pathways with fungal networks only, as fungi are
317 well-known for their crucial role in decomposing plant litter[22,23]. Furthermore, plant species litter
318 attracts specific fungal decomposers[22,23], which could explain why fungal networks were particularly
319 linked to past compositional pathways in which plant identity plays a key role. Additionally, studies
320 demonstrating a successional transformation of fungal communities from early to late decomposers
321 suggests that changes in litter input may result in long-lasting effects[22,23]. We conclude that litter input
322 and decomposition processes likely create long-lasting soil legacies critical for the decoupling of
323 prokaryote and fungal networks, which ensure network stability (Fig 7).
324 We further found that putative plant pathogens were consistently associated with past plant
325 community pathways in natural grassland soil. Soil-borne pathogens are increasingly found to be key
326 to plant stabilising processes by preventing dominant plant species from outcompeting less successful

327 plants[2–4]. In line, we found specific network clusters of putative pathogens to be increased when early
328 plant species maintained abundant after the start plant invasion in 2012. Studies support the idea that
329 negative soil legacy effects increase in strength with plant species time since establishment in a new
330 range[24,25] and similar temporal processes have been suggested to occur within plant communities
331 itself[26]. Additionally, we found generalist pathogens to be increased when plant species with a high
332 soil resource optima became more abundant with the start of invasion. This relation aligns with the
333 well-established trade-off between plant species growth and defence, where species with high soil
334 resource needs prioritise growth over defence[27]. Importantly, the specialisation of putative plant
335 pathogens towards various past pathways was absent in abandoned arable soil. Instead, almost all
336 putative plant pathogens were found in a single cluster and associated with a single past pathway. We
337 therefore conclude that plant-mediated soil legacy effects via soil-born pathogen accumulation is
338 likely to play an essential role in ensuring plant community stability.
339
340 *Soil chemistry is a critical mediator in coupling microbial networks*
341 We show that soil chemistry is a key mediator in plant-microbiota interactions. Interestingly, the
342 position of soil chemistry in mediating plant community pathways differed between abandoned arable
343 and natural grassland soil. In abandoned arable soil, soil chemical changes largely associated with
344 plant community pathways in the year of sampling, while in natural grassland soil, past plant
345 community pathways largely mediated soil chemical changes. This difference likely resulted from the
346 varying extent of soil resource depletion in the two soils. Natural grassland soil was more depleted in
347 N and organic C than abandoned arable soil, both at the start of the experiment and after 13 growing
348 seasons[18]. This N depletion leads to a shift towards using more recalcitrant C by soil microbes, causing
349 soil C depletion and a substantial increase in microbial network complexity[28]. Consequently, plants in
350 the year of sampling could have little impact on overall soil chemistry and past litter inputs created
351 strong legacies in natural grassland soil. This is in line with the findings that plant composition in the
352 year of sampling associated with microbial communities either through belowground productivity or
353 direct pathways, which both likely resulted from interactions with plant rhizodeposits such as root
354 exudates[29]. We suggest that soil resource depletion plays a major role in decoupling fungal from
355 prokaryote responses by enhancing soil legacy effects.
356 Contrary to natural grassland soil, plant composition in the year of sampling had a significant
357 impact on soil chemistry in abandoned arable soil. These changes in soil chemistry associated with
358 both prokaryote and fungal networks, likely coupling their responses. We found that soil N cycling was
359 increased with a high cover of legumes and a high cover of high resource optima species (mainly
360 invaded plant species). Legumes are well-known to enrich N levels in soil through N-fixation, but

361 increased soil N is also commonly observed when ruderal plant species invade communities[20,30]. In the
362 latter case, observed associations with ammonia oxidising archaea implies a crucial role for plant-
363 controlled processes such as rhizosphere priming - the acceleration of organic matter turnover via
364 root exudation - in enhancing soil N cycling and coupling prokaryote and fungal networks[31,32]. Notably,
365 these same plant compositional pathways were correlated with a reduction in fungal specialisation
366 and biomass, implying direct detrimental effects on fungal community complexity. We conclude that
367 soil chemistry and the ability of plants to influence soil resource cycling are crucial mediators in the
368 coupling/decoupling of microbial networks.
369
370 *Plant diversity decouples soil microbial networks in natural grassland soil*
371 We show that plant diversity in the year of sampling was a key factor in shaping prokaryotes and fungal
372 communities in abandoned arable soil. In addition to reducing bacterial, fungal and AMF biomass,
373 plant diversity was associated with the dominant prokaryote and fungal network clusters, thus likely
374 coupling their responses. In contrast, in natural grassland soil, plant diversity increased fungal diversity
375 and directly associated with fungal clusters only. Given that we took six soil cores from each mesocosm
376 and pooled these, it is likely that plant diversity pathways related to the extent of spatial heterogeneity
377 of the communities. Diverse plant communities are inherently more spatially heterogenous above-
378 and belowground, and this is likely to result in higher fungal diversity, as different plant species have
379 unique associated fungi[23,33]. In other words, a higher fungal diversity would be observed when
380 sampling at multiple spaces in a diverse plant community compared to a low diverse community.
381 Importantly, spatial soil heterogeneity has been suggested to be critical in ensuring diversity and
382 stability in plant communities[2,26,34]. We suggest that plant diversity is critical to stability by creating
383 spatial soil heterogeneity and decoupling prokaryote and fungal networks (Fig 7).
384 Our findings in abandoned arable soil suggest that plant diversity did not lead to the same level of
385 spatial heterogeneity as observed in natural grassland soil. To understand this, it is important to realise
386 that plant diversity differences in our communities resulted from local plant species loss and invasion
387 processes over time. As these processes are non-random, a low plant diversity likely represented a
388 situation in which certain successful plant strategies dominated, resulting in low plant functional
389 diversity and low soil multifunctionality[35,36]. Hence, plant species in low diverse assemblies on
390 abandoned arable soil likely affected microbial communities in relative similar ways, resulting in a
391 coupling of prokaryote and fungal responses, and instable networks (Fig 7).
392

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 suggest that interactions of microbiota with plants and soil resource cycling underlie the decoupling of prokaryote and fungal networks. Plant community and soil chemical parameters therefore likely provide important, easy to measure predictors of belowground microbial network stability of grasslands globally[5]. Similarly, plant community and soil chemical factors are promising aspects to consider in designing plant communities with decoupling effects on soil microbial networks to increase stability of agricultural systems[37,38]. Future challenges 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[5,39].

Methods

Experimental design

Experimental plant communities consisting of 44 perennial dry grassland species were sown in May 2007 (Table S9)[18]. Plant species were sown in equal proportions with three sowing densities on two soil origins: 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 grassland 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 S13A). 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[18]). Despite significant effects of sowing density in the first three years of the experiment[18], 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[18]. 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.

426 Importantly, mesocosms were regularly inspected and all species from outside the sown species
427 pool were weeded until September 2011. After this, plant species from the experimental surroundings
428 of the mesocosms were allowed to invade into the established plant communities (Fig S13B; Table S9).
429
430 *Plant species aboveground proportions*
431 Plant community aboveground biomass was harvested every July and September from 2007 until
432 2011. From 2012 onward, aboveground biomass was harvested only once a year in July. These time
433 points are similar to management practises at the natural grassland site. Aboveground biomass was
434 cut off 3 cm above the soil and from 2007 until 2011, biomass was sorted per plant species, dried at
435 60 ℃ for at least 48 h, after which dry weight was determined. From 2012 onward, plant species
436 biomass was estimated by determining the percentile abundance of each plant species per mesocosm
437 and multiplying this by the total aboveground biomass cut at 3 cm height and dried at 60 ℃ for at least
438 48 h. In 2014 and 2015, aboveground biomass was cut, but no species proportions were determined.
439
440 *Soil sampling*
441 After the growing season in December 2019, soil cores of 6 cm in diameter and 36 cm length were
442 taken at six random positions in each plant community (Fig S13B). Aboveground plant parts were
443 removed and soil of the six cores was thoroughly mixed by passing it through a 2 mm mesh. Roots that
444 did not pass the mesh were collected, dried at 60 ℃ for at least 48 h after which dry weight was
445 determined. Subsamples from the mixed soils were taken for soil chemical determination of total
446 nitrogen (N), total and organic C, plant available NO_3^-, NH_4^+ and NO_2^-, K, P and pH (see Supplementary
447 methods). Furthermore, subsamples for analyses of total bacterial, fungal and arbuscular mycorrhizal
448 fungi (AMF) biomass using PLFA and NLFA analyses were taken as well as for microbiome community
449 composition analyses using 16S and ITS amplicon sequencing (see Supplementary methods).
450
451 *Plant community diversity and composition*
452 All analyses were performed in R version 3.6.1[40]. Plant community diversity was calculated based on
453 the Shannon index using the function *diversity* of the vegan package[41]. Plant community temporal
454 stability was calculated based on the inverse coefficient of variation of total aboveground productivity
455 over time using the package codyn[42]. The first year of data was removed for this calculation as the
456 communities were still establishing, which resulted in a disproportionally large fluctuation in
457 aboveground productivity (Fig 1B).
458 Plant community compositional changes were assessed using detrended correspondence analysis
459 (DCA). For this, aboveground biomass of species with in total > 10 observations over all replicates and

460 all years was analysed using *decorana* from the vegan package[41]. To understand to which general
461 processes the compositional changes associated, we created a passive overlay of plant species
462 ecological optima (Czech Ellenberg indicator values for soil nitrogen, moisture and reaction[43]) and
463 plant species average residence period (average year of presence between 2012 and 2019) for DCA
464 scores 1 and 2 using *envfit* of the vegan package[41] (Fig S1A). Permutations (999 permutations) were
465 used to test for significance. DCA score 3 was not significantly related to any plant traits (data not
466 shown), but to a change in legume cover (Fig S1B).
467
468 *Past plant community parameters*
469 To describe variation in plant community development over time, we calculated two types of past
470 plant community parameters: the initial impact of the start of plant species invasion on the plant
471 community and developmental trajectories after the start of invasion. These two types of past
472 parameters were calculated for plant community aboveground productivity, plant diversity (Shannon
473 index) and the DCA scores 1 to 3. For the initial impact of plant invasion, we calculated the difference
474 between parameters values in 2011 (last year without invasion) and 2012 (first year with invasion).
475 This was with exception of plant diversity for which drastic changes due to the start of invasion
476 occurred over a period of two years and the one-year difference did not relate significantly to any soil
477 or microbial measurements in 2019 (data not shown). Initial invasion impact for plant diversity was
478 therefore calculated between 2011 and 2013. Developmental trajectories were calculated as overall
479 increase/decrease trends of each plant community parameter from 2012 until 2019. For this, we fitted
480 a linear model using *lm* and extracted the beta slope using *lm.beta*[40].
481
482 *16S and ITS bioinformatics*
483 In total, Illumina paired end sequencing of 120 samples (60 16S and 60 ITS amplicons) and 2 negative
484 controls yielded 4 261 236 raw sequences. The data were processed using the pipeline SEED2 ver.
485 2.1.1b[44]. First, low-quality sequences were discarded (mean quality score < 30). The reads were
486 demultiplexed (no mismatch allowed in the tag sequences) and tag switches (i.e. reads with non-
487 matching tags) were discarded.
488 The ITS2 region was extracted from the fungal amplicons using ITSx ver. 1.0.11[45] and sequences
489 shorter than 20 bp were excluded. This yielded 982 036 sequences which were clustered to OTUs using
490 UPARSE implementation in USEARCH ver. 8.1.1861[46] with 97% similarity threshold (45 480 chimeric
491 sequences were excluded during this step). The most abundant sequences were selected for each of
492 the resulting 10 685 OTUs. These sequences were checked for their identity via BLASTn algorithm
493 against the UNITE database ver. 8.3[47] and non-fungal, no-hit sequences as well as global singletons,

494 doubletons and tripletons were excluded from further analyses leaving 2638 OTUs represented by 840
495 206 reads. Six reads detected in the negative control were subtracted from the corresponding two
496 OTUs across the dataset. The ecological guilds of the fungal OTUs were parsed using the database
497 FungalTraits[48] at genus and sequence levels. All abundant OTUs were assigned with high probability.
498 Primers were cut from prokaryote reads (1 319 594 reads after demultiplexing) and sequences
499 shorter than 20 bp were excluded. The reads were clustered to OTUs using UPARSE implementation
500 in USEARCH ver. 8.1.1861[46] with 97% similarity threshold (442 826 chimeric sequences were excluded
501 during this step). OTUs with n < 5 were discarded. The most abundant sequences were selected for
502 each of the resulting 6532 OTUs. These sequences were checked for their identity via BLASTn
503 algorithm against the RDP trainset 16[49]. 179 reads detected in the negative control were subtracted
504 from the corresponding OTUs across the dataset. OTUs with non-target and no BLASTn hits were
505 excluded from further analyses leaving 6369 OTUs represented by 841 512 reads.
506
507 *Microbial community analyses*
508 Significant separation between soil origins was tested with PERMANOVA using *adonis* from the vegan
509 package[41] based on centered log ratio (clr) transformed read counts and visualised using PCA with the
510 phyloseq package[50]. Alpha-diversity (Shannon diversity) was calculated based on multiple rarefaction
511 (1000 iterations) using the metagMisc package[51]. The overlap in OTUs between natural grassland and
512 abandoned arable soil for prokaryotes and fungi was calculated using *ps_venn*[52].
513 For each OTU, we calculated a specialisation index (SI)[53]. SI of the *i*th OTU was calculated as the
514 coefficient of variation minus a correction for under-sampling of rare OTUs:

515 $$SI_i = \left(\frac{\sigma_i}{\mu_i}\right) - \sqrt{\frac{K}{N_i}}$$

516 with σ_i being the standard deviation of the reads of the *i*th OTU across all samples, μ_i being the mean
517 of the reads of the *i*th OTU across all samples, K the number of habitat classes (2 soils) and N the total
518 number of reads of the *i*th OTU across all samples. Calculations were based on rarefied read
519 abundances (rarefied to the smallest sample size: 4517 and 1618 for 16S and ITS, respectively).
520 Average SI of each sample was calculated as the community weighted mean of the SI of all OTUs
521 present in the sample.
522
523 *Microbial network analysis*
524 We constructed co-occurrence networks across the 30 plant communities on each soil origin for both
525 prokaryote (bacteria and archaea) and fungal communities. We first filtered each dataset to exclude
526 rare OTUs with total < 100 reads and OTUs that were present in < 5 samples per soil origin. Co-

527 occurrence networks were then calculated using the SpiecEasi package[54]. In brief, networks were
528 inferred based on clr transformed read counts, neighbourhood selection (MB method) and we
529 selected optimal stability parameters using the StARS selection approach (threshold 0.05, nlambda 20
530 with 999 replications)[55]. We clustered similarly responding OTUs in each network using the Spin-glass
531 algorithm of the igraph package[56–59]. This approach clusters OTUs based on both positive and negative
532 edges as well as their weight. Present and absent edges as well as positive and negative edges were
533 given a similar importance, and unlimited spins (clusters) were provided. Relative read counts were
534 summed per cluster per sample and used in further correlation and structural equation models (SEM).
535 We tested whether networks were (i) significantly more or less densely clustered, and (ii)
536 significantly more or less coupled in prokaryote and fungal clusters than based on chance using a null-
537 model approach. For this, we rewired the original networks edges with preserving the original
538 networks degree distribution using the robin package[60]. We (i) calculated the clustering coefficient
539 (modularity), and (ii) the number of significant correlations occurring between prokaryote and fungal
540 clusters of 1000 rewired networks. *P*-values were calculated as the proportion of randomised (i)
541 clustering coefficients and (ii) number of significant correlations smaller than in the original
542 networks[3,61].
543 The coupling/decoupling of prokaryotes and fungi *within* each plant community was described by
544 the beta slope of a generalised linear model (glm) between the summed relative read counts in the
545 most prevalent prokaryote 1-9 and fungal clusters 1-9. This correlation is valid as cluster assignment
546 within the microbial networks is based on the order of prevalence of the ASVs in each cluster, hence
547 the similarity in patterns of cluster sizes that occurred across different networks (Fig 3AB). Summed
548 relative read counts per cluster were ln- or sqrt-transformed when model residuals did not follow a
549 normal distribution. The beta slope was extracted using *lm.beta*[40]. A positive value indicates that
550 prokaryote and fungal reads were distributed similarly over the most prevalent network clusters, and
551 thus, that prokaryote and fungal responses were coupled. A value of zero indicates that prokaryote
552 and fungal read distribution over the network clusters was unrelated and thus decoupled. A few weak,
553 negative correlations occurred and indicate that prokaryote and fungal read distribution over the
554 network clusters were nearing an opposite pattern. These indicated cases of a strong decoupling of
555 prokaryote and fungal responses.
556
557 *Microbial network cluster characteristics*
558 We inferred putative dominant metabolic and functional characteristics of each microbial network
559 cluster. This was done based on similarities, if present, in knowledge of the prokaryote or fungal taxa
560 co-occurring in each network cluster: putative functions were obtained from literature and the

561 FungalTraits database[48], relative habitat specialisation, and enrichment in natural grassland or
562 abandoned arable soil (Fig S9-S10; Table S2-S5). Relative habitat specialisation of network clusters was
563 calculated by taking the community weighted mean of the SI of each sample in each cluster (see
564 Microbial community analysis). Relative habitat generalist clusters were defined as clusters below the
565 community-wide mean SI without overlap of the maximum distribution (75th percentile + 1.5 *
566 interquartile range). Relative habitat specialists were defined as clusters above the community-wide
567 mean SI without overlap of the minimum distribution (25th percentile - 1.5 * interquartile range) (Fig
568 S9)[53].
569 Cluster enrichment in either of the soils was calculated by taking the ln-response ratio of read
570 counts (rarefied) in natural grassland soil divided by read counts in abandoned arable soil for each
571 OTU. Network clusters with response ratios above zero and without overlap of the minimum
572 distribution were defined as enriched in natural grassland soil. Network clusters with response ratios
573 below zero and without overlap of the maximum distribution were defined as enriched in abandoned
574 arable soil (Fig S10).
575
576 *Overall statistics*
577 Analysis of variances were performed using linear mixed-effects models in R version 3.6.1[40]. All models
578 in which 'time' (continuous, scaled) and 'soil origin' were included as an explanatory variable, included
579 'mesocosm' and 'sowing density' as random effects to take repeated measures and initial sowing
580 densities into account. Models were fitted using *lmer* of the lme4 package[62]. Mesocosm position in
581 the garden had negligible effects, in many cases resulted in overfitting of the models and was
582 therefore dropped as a random effect.
583 Correlations between plant, soil chemistry and microbial parameters including network clusters
584 were performed using *lme* of the nlme package[63] and included sowing density as a random factor. For
585 these multiple correlations, p-values were corrected for multiple testing using the Bonferroni
586 correction[64]. In all models, data was ln- or sqrt-transformed when model residuals did not follow a
587 normal distribution. In case of heterogeneity of variances, data weighting per soil origin using *varIdent*
588 from the nlme package was incorporated[65]. Following Tukey HSD post-hoc tests were performed using
589 the package emmeans[66].
590

591 *Structural equation modelling*

592 We hypothesised that plant community parameters in the year of sampling and developmental
593 trajectories in time affected microbial communities with soil chemical changes as a potentially
594 important mediator. We used structural equation models (SEM) which allowed us to separate direct
595 pathways between plant communities and soil microbiota from indirect pathways via soil chemical
596 changes (Fig 5A). We note that the directionality of soil chemistry onto microbial communities is a
597 mathematical necessity to test whether soil chemistry was a significant mediator. Such pathways
598 should however be interpreted as an interaction as both soil microbiota and plant communities
599 influence soil chemistry.

600 All SEMs were fit using piecewiseSEM[67] and *lme* of the nlme package[63] with initial sowing density
601 as a random effect (n = 30). Overall model fit was assessed using direction separation tests (d-sep)
602 based on Fisher's C statistics with models being accepted if $p > 0.1$. We simplified our models using a
603 backward stepwise elimination procedure for which we consecutively removed pathways with the
604 highest p-value[68]. Endogenous variables were allowed to drop from the model in case effects were
605 not significant ($p > 0.05$). The model with the lowest Akaike information criterion (corrected for small
606 sample sizes; AICc) was then selected as the best fit base model (see Supplementary Methods).

607 We created 64 unique SEM models (3 microbial biomass pools natural grassland soil + 3 microbial
608 biomass pools abandoned arable soil + 9 16S clusters natural grassland soil + 10 16S clusters
609 abandoned arable soil + 21 ITS clusters natural grassland soil + 18 ITS clusters abandoned arable soil).
610 We extracted the direction and effect size of each significant pathway of each model ($p < 0.05$) and
611 calculated the contribution of each plant parameter to changes in microbial biomass and microbial co-
612 occurrence clusters. For each significant, direct plant-microbial pathway, we multiplied the path effect
613 size with the relative size of the microbial pool or cluster it was affecting. This multiplication sized the
614 pathway effect to its relative importance to the microbial community as a whole. For indirect
615 pathways, we multiplied the effect size of the plant parameter onto the soil chemical variable with
616 the effect size of the soil chemical variable onto the microbial variable. The obtained effect sizes were
617 scaled to the relative size of the microbial variable they were affecting using community weighted
618 means.

619 To create an overview of all effects, the relative contribution of each plant parameter per microbial
620 variable was summed per group: all combinations of plant parameters in the year of sampling versus
621 past parameters, overall versus compositional parameters, and direct versus indirect pathways (Fig
622 5A). These grouped effect sizes were scaled to the number of potential pathways within each group
623 to allow a direct comparison of the relative contribution of each plant parameter group (Fig 5B).

624

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Author contributions

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

Data availability

All data is publicly available via the Zenodo digital data repository: doi.org/10.5281/zenodo.6695065. Microbial sequencing data is deposited in the NCBI SRA database, BioProject PRJNA931221.

Code availability

All R scripts are publicly available via Github:
github.com/dintzandt/scripts_stability_and_soil_microbial_networks.

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