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 associated with soil microbial networks. Abandoned arable soil promoted destabilising properties and was associated with soil microbial networks coupled in prokaryote and fungal responses. This coupling was linked with strong associations between prokaryotes, fungi and the plant community with soil chemical composition. Conversely, in stable natural grassland communities, soil prokaryote and fungal responses were decoupled. This decoupling was associated with a large variety of past plant community pathways affecting in particular fungal networks. We conclude that plant community stability is associated with a decoupling of prokaryote and fungal soil networks in which interactions with the plant community play a critical role.

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 comprehensible 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 via, for example, 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 ofindirect plant community pathways via 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. 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[18,19]. 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[20]. 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 their role in 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 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, 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).

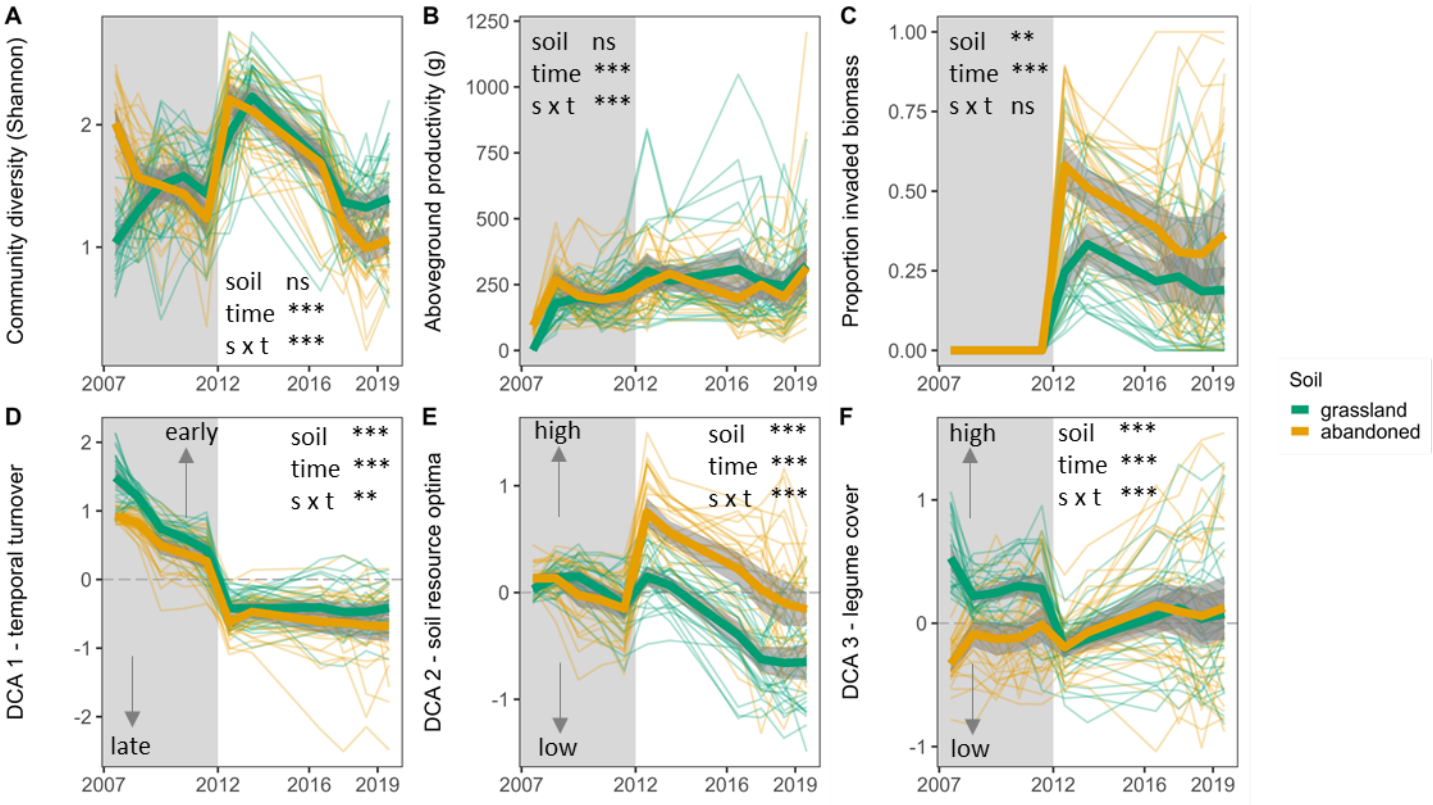

Figure 1 Plant community development over time on natural grassland (green) and abandoned arable soil (yellow). Plant community (A) diversity, (B) total biomass, (C) biomass proportion of invaded plant species, and community compositional DCA scores related to plant species (D) temporal turnover, (E) ecological soil resource optimum and (F) legume cover. In D-F 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). 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 1D-F, 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 1D, 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 1E, S1A). This increase was strongest for communities on abandoned arable

115 soil and persisted in the following years (Fig 1E, S1A). Finally, DCA 3 separated plant community
116 composition between the two soil origins before the start of invasion: communities on natural
117 grassland soil harboured a higher cover of legumes than communities on abandoned arable soil in the
118 years before 2012. With the start of invasion, plant community composition on natural grassland soil
119 dropped to, on average, similar legume cover as for the abandoned arable communities (Fig 1F, S1B).
120

121 *Soil origin affected soil chemistry and microbial soil communities*

122 After the 13th growing season, natural grassland and abandoned arable soils differed significantly in
123 chemistry, microbial biomass and microbial community composition (Fig 2AB, S2). Abandoned arable
124 soil was significantly higher in total N, plant available NO_3^-, NO_2^-, and NH_4^+, total and organic C and
125 total P. In addition, prokaryote and fungal richness as well as bacterial biomass were significantly
126 higher in abandoned arable than natural grassland soil (Fig S2). Conversely, soil pH, fungal and AMF
127 biomass, and belowground productivity were higher in natural grassland soil (Fig S2). Both prokaryote
128 and fungal community composition were significantly different between natural grassland and
129 abandoned arable soil (Fig 2AB). These differences largely resulted from differences in prokaryote and
130 fungal OTU abundances given the large overlap in the OTUs that were present in both soils (Fig S3AB).

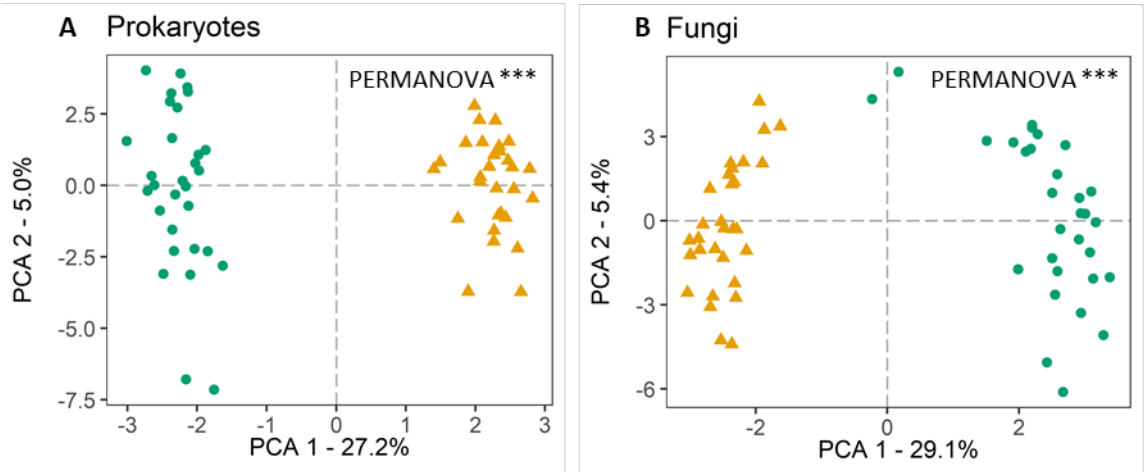

Figure 2 Principal Component Analysis (PCA) on clr-transformed (A) prokaryote 16S and (B) fungal ITS rRNA reads in plant communities established on natural grassland (green circles) and abandoned arable soil (yellow triangles). Amplicon sequencing was performed at the end of the 13th growing season (*n*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

131

132 *Microbial networks in natural grassland soil were decoupled in prokaryote and fungal responses*

133 For each soil origin, we created microbial co-occurrence networks. The networks indicated highly
134 connected prokaryote and fungal communities in both natural and abandoned soil with few
135 dominating OTUs and little difference in network properties commonly associated with network
136 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 would be expected based on null-models (randomly rewired networks; Fig S7), indicating a distinct organisational pattern of each network.

We summed the relative abundance of the OTUs in each cluster. On average, three dominant prokaryote clusters occurred that held most of the 16S rRNA reads recovered in both soils (Fig 3AB, S5AB). In abandoned arable soil, these patterns were similar for the fungal networks showing 3 large clusters. On natural grassland soil, however, fungal networks showed 5 larger clusters (Fig 3AB, S5CD).

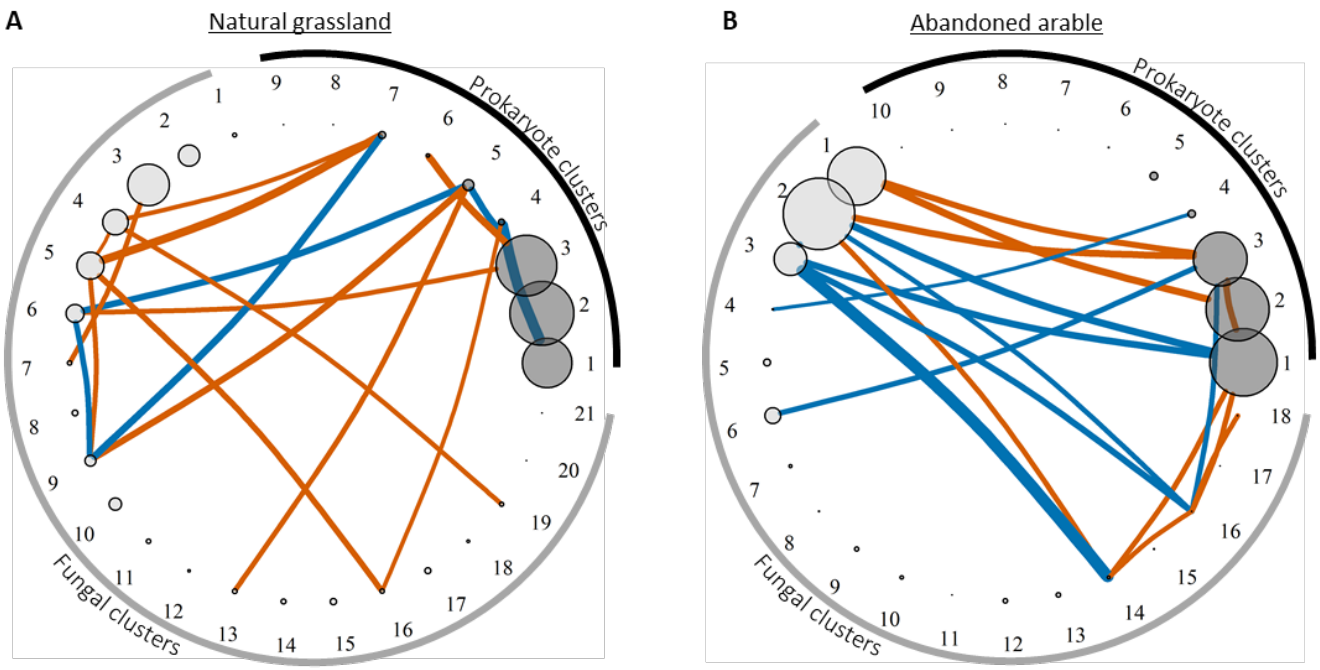

Figure 3 Correlations between prokaryote (dark grey) and fungal network clusters (light grey) in (A) natural grassland and (B) abandoned arable soil. Only significant correlations after Bonferroni correction for multiple testing are presented ($p < 0.0017$ and $p < 0.0018$ for natural grassland and abandoned 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.

We tested for correlations between all clusters in each soil origin. Most strikingly, in natural grassland soil, only a single correlation between the dominant prokaryote and fungal clusters occurred, while various strong positive and negative correlations occurred between the most

152 dominant clusters in abandoned arable soils (Fig 3AB). In other words, responses of dominant
153 prokaryote and fungal clusters in networks of natural grassland soil were decoupled, while in
154 abandoned arable soil, dominant prokaryote and fungal clusters responded in tandem.
155
156 *Microbial communities in abandoned arable soil were predominantly associated with the plant*
157 *community in the year of sampling*
158 We hypothesised that the plant community plays an important role in shaping soil microbial networks
159 and biomass, and may underlie the coupling/decoupling of prokaryote and fungal networks. We
160 tested this hypothesis using structural equation modelling (SEM). For each soil origin, we determined
161 how plant community parameters from the year of sampling and from the past (initial plant invasion
162 impact in 2012 and plant community developmental trajectories) associated with microbial biomass
163 and network clusters at the end of the 13th growing season (Fig 4A). We tested whether these
164 associations resulted from overall plant community factors (aboveground productivity and plant
165 diversity) or plant community composition (DCA scores), and determined whether these pathways
166 occurred via direct or indirectly pathways via soil chemical changes (Fig 4A). The obtained SEM models
167 explained, on average, 47% of the variation in microbial biomass. For microbial clusters, the explained
168 variation varied strongly between the network clusters in both soil origins and lay between 0 and 64%
169 (Table S6).
170 We calculated the relative contribution of each group of plant community parameters in shaping
171 soil microbial properties using the effect sizes of the SEM pathways. Most strikingly, we found that
172 plant community parameters in the year of sampling played a much larger role in shaping both
173 microbial biomass and networks in abandoned arable than natural grassland soil (Fig 4B).
174 Furthermore, the main players in the year of sampling in abandoned arable soil were direct
175 associations with the overall plant community and indirect associations of plant composition. In
176 contrast, in natural grassland soil, associations in the year of sampling most consistently occurred via
177 direct plant compositional pathways. Additionally, a few indirect plant compositional pathways
178 occurred for prokaryote and fungal network clusters, but not for microbial biomass. Direct
179 associations with the overall plant community occurred solely for fungal clusters (Fig 4B).
180 A variety of past plant community parameters were important in shaping microbial communities
181 in natural grassland soil. Microbial biomass was largely shaped by direct and indirect pathways of past
182 overall plant community factors. Prokaryote and fungal clusters were largely shaped by indirect
183 pathways of the past overall plant community as well as past direct compositional pathways (Fig 4B).
184 In abandoned arable soil, most of these past pathways were absent with exception of direct
185 compositional effects on prokaryote network clusters (Fig 4B).

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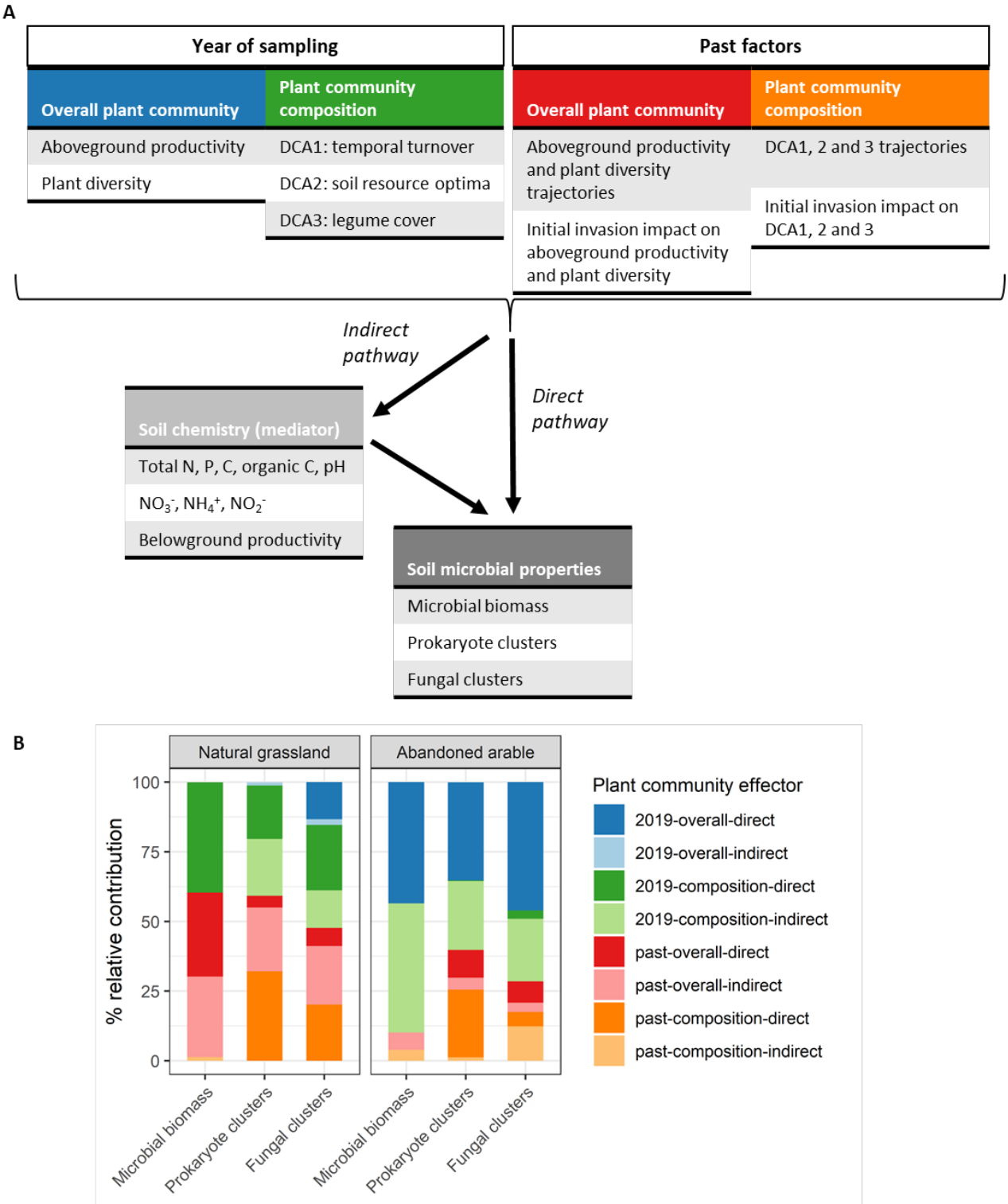

Figure 4 Structural equation modelling (SEM) coupling natural variation in plant communities, soil chemistry mediators (including plant belowground productivity effects), microbial biomass and microbial network clusters in plant communities established on natural grassland and abandoned arable soil. (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 indicate 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.

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

To compare the specific processes associated with prokaryote and fungal network clusters in natural grassland and abandoned arable soil, we extracted all direct and indirect plant community pathways with a relative contribution > 5%. We determined whether these pathways occurred for both prokaryotes and fungal clusters or were unique to either group (Fig 5AB).

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 5A). 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 5A).

In abandoned arable soil, less differentiation in past pathways between prokaryote and fungal networks occurred (Fig 5B). Some similarities to the natural grassland soil occurred, such as unique

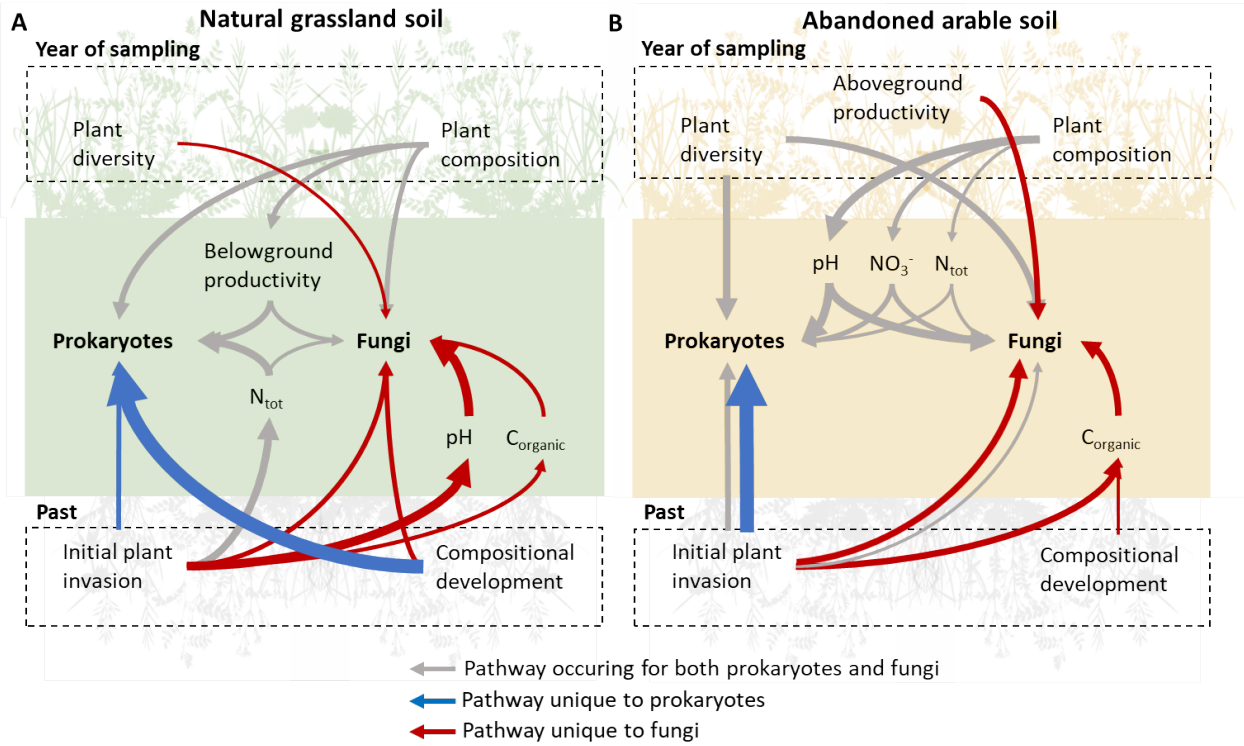

Figure 5 Summary of the most important pathways and plant community factors shaping prokaryote and fungal soil network clusters in (A) natural grassland soil and (B) abandoned arable soil. Grey arrows indicate pathways occurring for both prokaryote and fungal clusters, blue arrows pathways unique to 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.

202 pathways for prokaryote and fungal clusters resulting from the initial effect size of invasion in 2012
203 (Fig 5B). However, on abandoned arable soil, overlapping pathways associating with plant diversity
204 and composition in the year of sampling were more numerous than unique past pathways (Fig 4B).
205
206 *In the year of sampling, plant composition decreased fungal community specialisation in abandoned*
207 *arable soil*
208 As a final step, we searched for generalities in the prokaryote and fungal characteristics that were
209 associated with plant community pathways by testing which pathways significantly associated with
210 overall microbial community patterns: alpha-diversity (Shannon's index) and the relative habitat
211 specialisation index (SI). Additionally, we sought to understand the functional traits of each microbial
212 network cluster by analysing their taxonomic composition and comparing it to existing literature and
213 databases, and calculating the taxa's relative habitat specialisation and enrichment in natural
214 grassland or abandoned arable soil (Fig S8-S9; Table S2-S5).
215 In the year of sampling, we found that plant composition in the year of sampling did not affect any
216 of the soil nutrients in natural grassland soil, only belowground productivity was affected (Fig 5A; Table
217 S7). As a result, most plant compositional pathways in the year of sampling occurred via direct
218 interactions. A high legume cover in the year of sampling was associated with a low fungal and AMF
219 biomass, and plant communities with a high cover of high resource optima plant species were
220 associated with a low fungal diversity and bacterial biomass. In the latter case, belowground
221 productivity was low and therewith increased prokaryote diversity (Table S7). Furthermore, in
222 contrast to abandoned arable soil, plant diversity did not have detrimental effects on the fungal
223 community and rather increased fungal diversity in natural grassland soil (Table S7). Most of the
224 pathways in natural grassland soil involved metabolically diverse groups of bacteria, putative soil and
225 litter saprotrophs as well as plant pathogens (Table S7).
226 In abandoned arable soil, we found that plant diversity and composition in the year of sampling
227 associated with a large number of prokaryote and fungal network clusters. In contrast to natural
228 grassland soil, the plant compositional pathways occurred via changes in soil pH, total N and NO_3^- in
229 abandoned arable soil (Fig 5B). Most of these pathways involved dominant prokaryote and fungal
230 clusters, putative soil saprotrophs, plant pathogens, ammonia oxidising archaea (AOA) and
231 metabolically diverse bacteria (Table S8). Interestingly, a high cover of high resource optima plant
232 species and a low cover of legumes resulted in a low fungal community specialisation, which was
233 mediated through an increase in soil pH (Table S8). Additionally, a high legume cover and high plant
234 diversity decreased fungal biomass (Table S8; Fig S10B-S11).
235

236 *Past plant community pathways were especially associated with putative saprotrophs and pathogens*
237 In natural grassland soil, past plant community pathways were numerous and diverse, but for an
238 important part related to the initial impact that plant species invasion had on the plant communities
239 (Table S7; Fig S10). A large increase in aboveground productivity with the start of plant invasion in
240 2012 was associated with an increased soil N and decreased soil pH after the 13th growing season
241 (2019). Subsequently, fungal and bacterial biomass as well as prokaryote community specialisation
242 were increased. Similarly, a large increase in plant diversity with invasion in 2012 was associated with
243 a decreased soil organic C (Table S7; Fig S11). These past plant community pathways involved a large
244 diversity of microbiota, but especially diverse groups of chemoheterotrophic bacteria, putative soil
245 saprotrophs and plant pathogens (Table S7).
246 Importantly, in natural grassland soil, large changes in plant composition with the start of plant
247 invasion affected many fungal, but not prokaryote clusters. Prokaryote clusters were instead
248 associated with plant compositional trajectories over time (Fig 5A; Table S7). The various fungal
249 clusters affected by plant invasion impact especially consisted of putative soil saprotroph and plant
250 pathogens (Table S7). For this latter group, plant communities where few early plant species were lost
251 with the onset of plant invasion were increased in a specific set of putative soil pathogens. In addition,
252 communities with a large increase in plant species with high soil resource optima were increased in a
253 set of generalist soil pathogens, but decreased in another set of putative pathogens (Table S7).
254 In abandoned arable soil, past plant community pathways also occurred for an important part via
255 the initial impact of plant species invasion in 2012. These pathways, however, consistently affected
256 both prokaryote and fungal clusters and generally affected especially diverse groups of prokaryotes
257 as well as putative soil saprotrophs and plant pathogens (Fig 5B; Table S8). Interestingly, almost all
258 affected plant pathogens occurred in a single, dominant cluster, which was mediated by changes in
259 soil organic C (Table S8).
260
261 **Discussion**
262 *Plant community stability is associated with a decoupling in prokaryote and fungal responses*
263 We tested to what extent and via which pathways plant communities shaped end of season soil
264 microbial networks. We compared these pathways between dry grassland communities established
265 on natural grassland soil and soil abandoned from agricultural practices 60 years before the start of
266 the 13-year long mesocosm experiment. Our findings show that abandoned arable soil had
267 destabilising effects on plant-soil-microbial networks, compared to the natural grassland soil. This
268 instability was demonstrated by a higher success of invading plants in communities grown on
269 abandoned arable soil, which is in line with previous studies[18,19]. Furthermore, our results show that

270 this instability above ground was reflected in the soil microbial networks occurring after the 13th
271 growing season. In abandoned arable soil, the dominant fungal and prokaryote network clusters had
272 strong positive and negative co-occurrences, indicating a joint response to environmental factors. This
273 response was tied to the plant community, as both the prokaryote and fungal clusters were associated
274 with relative similar pathways related to plant-soil interactions. These patterns are characteristic for
275 instable networks, as perturbation effects can easily spread throughout the network, affecting large
276 proportions of the network rather than just a small part (Fig 6)[11,21]. This highlights the sensitivity of
277 these abandoned arable microbial networks to disturbances and the potential for major restructuring.
278 In contrast, we found that the prokaryote and fungal responses in natural grassland soil were
279 largely decoupled. The prokaryote and fungal networks clusters were, for an important part,
280 associated to separated pathways related to the plant community. This division likely created separate
281 soil niches for each group[22,23], leading to enhanced resilience against disturbances in the system. This
282 decoupled structure of microbial networks is associated with the ability to buffer against the spread
283 of disturbances: a disturbance affecting one or a few of the microbial clusters will not cascade into
284 affecting unconnected clusters (Fig 6; compartmentalization[11,21]). We conclude that plant community
285 stability is associated with a decoupling of prokaryote and fungal responses, which is likely shaped by
286 interactions with the plant community.

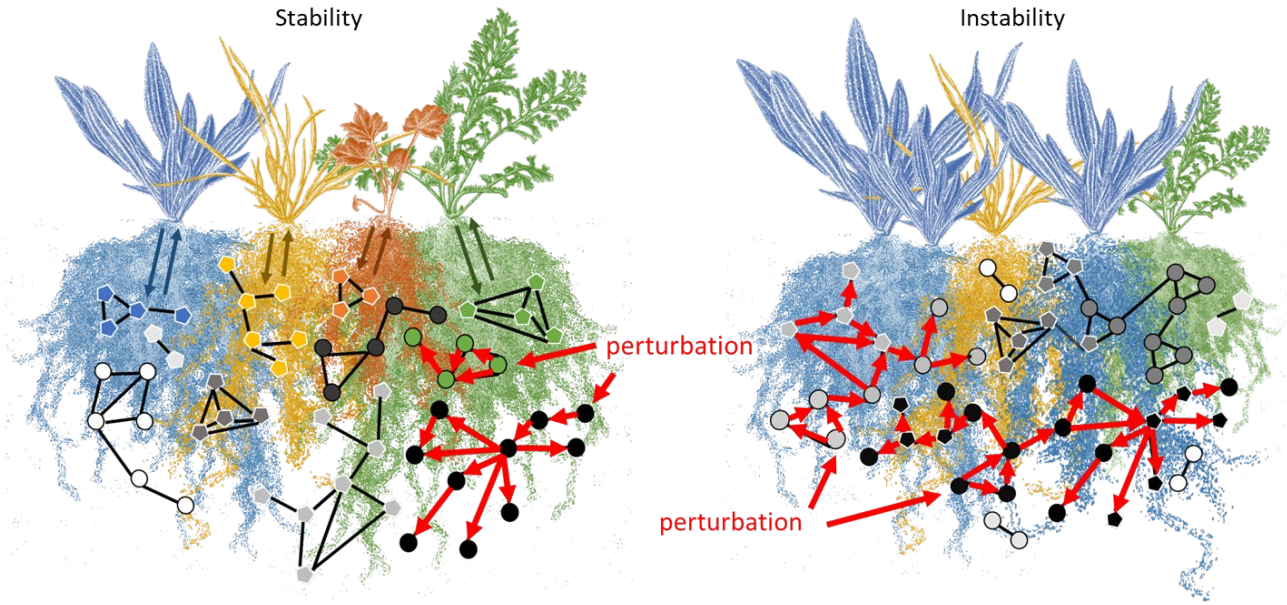

Figure 6 Conceptual framework reconciling the importance of the decoupling of prokaryote and fungal responses in buffering the propagation of local perturbation effects. In (A) stable settings, prokaryotes (circles, black border) and fungi (pentagons, white border) occur in separate clusters. This separation results from different factors shaping prokaryote and fungal niches. For example, fungal clusters are for an important part 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).

287 *Plant-mediated soil legacies play a key role in decoupling microbial networks*

288 We show that the soil microbial networks in natural grassland soil were more strongly associated with
289 past plant community pathways than the networks in abandoned arable soil. These past plant
290 community pathways suggested to separate the responses of prokaryote and fungal network clusters
291 in natural grassland soil. This suggests that plant-mediated soil legacies play a crucial role in decoupling
292 fungal and prokaryote responses, resulting in a more stable soil microbial community (Fig 6).

293 Plant-mediated soil legacies are well-known phenomena and play a driving role in plant community
294 coexistence, diversity and succession[2–4]. We found that plant-mediated soil legacies resulted, for an
295 important part, from the initial impact of plant species invasion in 2012. In natural grassland soil,
296 drastic increases in plant diversity, aboveground productivity and changes in plant composition
297 resulted in numerous changes in putative fungal saprotrophs after the 13th growing season. Together
298 with the lasting impact of the initial plant invasion on soil organic C, pH, and total N, our findings
299 suggest that past invasion pathways were largely due to changes in decomposition processes. This is
300 consistent with the observed association of these pathways with fungal networks only, as fungi are
301 well-known for their crucial role in decomposing plant litter[24,25]. Furthermore, plant species litter
302 attracts specific fungal decomposers[24,25], which could explain why fungal networks were particularly
303 linked to past compositional pathways in which plant identity plays a key role. The successional
304 transformation of fungal communities from early to late decomposers suggests that changes in litter
305 input may result in long-lasting effects[24,25]. We conclude that litter input and decomposition processes
306 likely create long-lasting soil legacies, critical for the decoupling of prokaryote and fungal networks,
307 which ensure network stability (Fig 6).

308 We further found that putative plant pathogens were consistently associated with past plant
309 community pathways in natural grassland soil. Soil-borne pathogens are increasingly found to be key
310 to plant stabilising processes by preventing dominant plant species from outcompeting less successful
311 plants[2–4]. In line, we found specific network clusters of putative pathogens to be increased when early
312 plant species maintained abundant after the start plant invasion in 2012. Studies support the idea that
313 negative soil legacy effects increase in strength with plant species time since establishment in a new
314 range[26,27] and similar temporal processes have been suggested to occur within plant communities
315 itself[28]. Additionally, we found generalist pathogens to be increased when plant species with a high
316 soil resource optima became more abundant with the start of invasion. This relation aligns with the
317 well-established trade-off between plant species growth and defence, where species with high soil
318 resource needs prioritise growth over defence[29]. Importantly, the specialisation of putative plant
319 pathogens towards various past pathways was absent in abandoned arable soil. Instead, almost all
320 putative plant pathogens were found in a single cluster and associated with a single past pathway. We

321 therefore conclude that plant-mediated soil legacy effects via soil-born pathogen accumulation is
322 likely to play an essential role in ensuring plant community stability.
323
324 *Soil chemistry is a critical mediator in coupling microbial networks*
325 We show that soil chemistry is a key mediator in plant-microbiota interactions. Interestingly, the
326 position of soil chemistry in mediating plant community pathways differed between abandoned arable
327 and natural grassland soil. In abandoned arable soil, soil chemical changes largely associated with
328 plant community pathways in the year of sampling, while in natural grassland soil, past plant
329 community pathways largely mediated soil chemical changes. This difference likely resulted from the
330 varying extent of soil resource depletion in the two soils. Natural grassland soil was more depleted in
331 N and organic C than abandoned arable soil, both at the start of the experiment and after 13 growing
332 seasons[20]. This N depletion leads to a shift towards using more recalcitrant C by soil microbes, causing
333 soil C depletion and a substantial increase in microbial network complexity[30]. Consequently, plants in
334 the year of sampling could have little impact on overall soil chemistry and past litter inputs created
335 strong legacies in natural grassland soil. This is in line with the findings that plant composition in the
336 year of sampling associated with microbial communities either through belowground productivity or
337 direct pathways, which both likely resulted from interactions with plant rhizodeposits such as root
338 exudates[31]. We suggest that soil resource depletion plays a major role in decoupling fungal from
339 prokaryote responses by enhancing soil legacy effects.
340 Contrary to natural grassland soil, plant composition in the year of sampling had a significant
341 impact on soil chemistry. These changes in soil chemistry associated with both prokaryote and fungal
342 networks, likely coupling their responses. We found that soil N cycling was increased with a high cover
343 of legumes and a high cover of high resource optima species (mainly invaded plant species). Legumes
344 are well-known to enrich N levels in soil through N-fixation, but increased soil N is also commonly
345 observed when ruderal plant species invade communities[19,32]. In the latter case, observed associations
346 with ammonia oxidising archaea implies a crucial role for plant-controlled processes such as
347 rhizosphere priming - the acceleration of organic matter turnover via root exudation - in enhancing
348 soil N cycling and coupling prokaryote and fungal networks[33,34]. Notably, these same plant
349 compositional pathways were correlated with a reduction in fungal specialisation and biomass,
350 implying direct detrimental effects on fungal community complexity. We conclude that soil chemistry
351 and the ability of plants to influence soil resource cycling are a crucial mediators in the
352 coupling/decoupling of microbial networks.
353

354 *Plant diversity decouples soil microbial networks in natural grassland soil*

355 We show that plant diversity in the year of sampling was a key factor in shaping prokaryotes and fungal
356 communities in abandoned arable soil. In addition to reducing bacterial, fungal and AMF biomass,
357 plant diversity was associated with the dominant prokaryote and fungal network clusters, thus likely
358 coupling their responses. In contrast, in natural grassland soil, plant diversity increased fungal diversity
359 and directly associated with fungal clusters only. Given that we took six soil cores from each mesocosm
360 and pooled these, it is likely that plant diversity pathways related to the extent of spatial heterogeneity
361 of the communities. Diverse plant communities are inherently more spatially heterogenous above-
362 and belowground, and this is likely to result in higher fungal diversity, as different plant species have
363 unique associated fungi[25,35]. In other words, a higher fungal diversity would be observed when
364 sampling at multiple spaces in a diverse plant community compared to a low diverse community.
365 Importantly, spatial soil heterogeneity has been suggested to be critical in ensuring diversity and
366 stability in plant communities[2,28,36]. We suggest that plant diversity is critical to stability by creating
367 spatial soil heterogeneity and decoupling prokaryote and fungal networks (Fig 6).

368 Our findings in abandoned arable soil suggest that plant diversity did not lead to the same level of
369 spatial heterogeneity as observed in natural grassland soil. To understand this, it is important to realise
370 that plant diversity differences in our communities resulted from local plant species loss and invasion
371 processes over time. As these processes are non-random, a low plant diversity likely represented a
372 situation in which certain successful plant strategies dominated resulting in low plant functional
373 diversity and low soil multifunctionality[37,38]. Hence, plant species in low diverse assemblies on
374 abandoned arable soil likely affected microbial communities in relative similar ways, resulting in a
375 coupling of prokaryote and fungal responses, and instable networks (Fig 6).

376

377 **Conclusion**

378 We found remarkably different topologies in soil microbial networks in plant communities established
379 on natural grassland soil and plant communities established on soil abandoned from extensive
380 agricultural practices 60 years before the start of the experiment. Microbial networks in stable natural
381 grassland soil were largely decoupled in prokaryote and fungal responses, while prokaryote and fungal
382 networks in instable abandoned arable soil largely responded in tandem. We suggest that interactions
383 of microbiota with plants and soil resource cycling underlie the decoupling of prokaryote and fungal
384 networks. Plant community and soil chemical parameters therefore likely provide important, easy to
385 measure predictors of belowground microbial network stability of grasslands globally[5]. Similarly, plant
386 community and soil chemical factors are promising aspects to consider in designing plant communities
387 with decoupling effects on soil microbial networks to increase stability of agricultural systems[39,40].

388 Future challenges lie in connecting plant and microbial community stability to its driving forces across
389 a multitude of ecosystems, soil resource conditions, land management and perturbation types[5,41].
390
391 **Methods**
392 *Experimental design*
393 Experimental plant communities consisting of 44 perennial dry grassland species were sown in May
394 2007 (Table S9)[20]. Plant species were sown in equal proportions with three sowing densities on two
395 soil origins: a dry natural grassland soil (excavated near Encovany, Czech Republic; 50°31'44.6"N,
396 14°15'12.6"E) and a soil on which dry natural grasslands was turned into agricultural land, extensively
397 managed and abandoned 60 years before soil was collected (excavated near Institute of Botany, Czech
398 Academy of Sciences; 50°0'7.11"N, 14°33'20.66"E) (*n* = 10, 60 plant communities in total; Fig S12A).
399 The two soils mainly differed in soil nutrient availability with the natural grassland soil being
400 significantly lower in total N, organic C and plant available P and K than the abandoned arable soil[20].
401 The three sowing densities represent 25%, 100% and 400% of the seed density per m^{-2} as estimated
402 at the natural dry grassland location (see Supplementary methods and[20]). Despite significant effects
403 of sowing density in the first three years of the experiment[20], sowing density did not significantly affect
404 above- and belowground plant, microbial and chemical properties in the current study (data not
405 shown). Sowing density was therefore incorporated as a random effect rather than a fixed factor in all
406 analyses.
407 Plant communities were grown in 90 L mesocosms (diameter 65 cm, height 36 cm) in the
408 experimental garden of the Institute of Botany, Czech Academy of Sciences[20]. This location provided
409 similar environmental conditions to the natural grassland location. Plant communities did not receive
410 any watering or fertiliser. Only in rare periods of extreme drought when plants showed signs of wilting,
411 communities were watered with rain water.
412 Importantly, mesocosms were regularly inspected and all species from outside the sown species
413 pool were weeded until September 2011. After this, plant species from the experimental surroundings
414 of the mesocosms were allowed to invade into the established plant communities (Fig S12B; Table S9).
415
416 *Plant species aboveground proportions*
417 Plant community aboveground biomass was harvested every July and September from 2007 until
418 2011. From 2012 onward, aboveground biomass was harvested only once a year in July. These time
419 points are similar to management practises at the natural grassland site. Aboveground biomass was
420 cut off 3 cm above the soil and from 2007 until 2011, biomass was sorted per plant species, dried at
421 60 ℃ for at least 48 h, after which dry weight was determined. From 2012 onward, plant species

422 biomass was estimated by determining the percentile abundance of each plant species per mesocosm
423 and multiplying this by the total aboveground biomass cut at 3 cm height and dried at 60 ˚C for at least
424 48 h. In 2014 and 2015, aboveground biomass was cut, but no species proportions were determined.
425
426 *Soil sampling*
427 After the growing season in December 2019, soil cores of 6 cm in diameter and 36 cm length were
428 taken at six random positions in each plant community (Fig S12B). Aboveground plant parts were
429 removed and soil of the six cores was thoroughly mixed by passing it through a 2 mm mesh. Roots that
430 did not pass the mesh were collected, dried at 60 ˚C for at least 48 h after which dry weight was
431 determined. Subsamples from the mixed soils were taken for soil chemical determination of total
432 nitrogen (N), total and organic C, plant available NO_3^-, NH_4^+ and NO_2^-, K, P and pH (see Supplementary
433 methods). Furthermore, subsamples for analyses of total bacterial, fungal and arbuscular mycorrhizal
434 fungi (AMF) biomass using PLFA and NLFA analyses were taken as well as for microbiome community
435 composition analyses using 16S and ITS amplicon sequencing (see Supplementary methods).
436
437 *Plant community diversity and composition*
438 All analyses were performed in R version 3.6.1[42]. Plant community diversity was calculated based on
439 the Shannon index using the function *diversity* of the vegan package[43]. Plant community compositional
440 changes were assessed using detrended correspondence analysis (DCA). For this, aboveground
441 biomass of species with in total > 10 observations over all replicates and all years was analysed using
442 *decorana* from the vegan package[43]. To understand to which general processes the compositional
443 changes associated, we created a passive overlay of plant species ecological optima (Czech Ellenberg
444 indicator values for soil nitrogen, moisture and reaction[44]) and plant species average residence period
445 (average year of presence between 2012 and 2019) for DCA scores 1 and 2 using *envfit* of the vegan
446 package[43] (Fig S1A). Permutations (999 permutations) were used to test for significance. DCA score 3
447 was not significantly related to any plant traits (data not shown), but to a change in legume cover (Fig
448 S1B).
449
450 *Past plant community parameters*
451 To describe variation in plant community development over time, we calculated two types of past
452 plant community parameters: the initial impact of the start of plant species invasion on the plant
453 community and developmental trajectories after the start of invasion. These two types of past
454 parameters were calculated for plant community aboveground productivity, plant diversity (Shannon
455 index) and the DCA scores 1 to 3. For the initial impact of plant invasion, we calculated the difference

456 between parameters values in 2011 (last year without invasion) and 2012 (first year with invasion).
457 This was with exception of plant diversity for which drastic changes due to the start of invasion
458 occurred over a period of two years and the one-year difference did not relate significantly to any soil
459 or microbial measurements in 2019 (data not shown). Initial invasion impact for plant diversity was
460 therefore calculated between 2011 and 2013. Developmental trajectories were calculated as overall
461 increase/decrease trends of each plant community parameter from 2012 until 2019. For this, we fitted
462 a linear model using *lm* and extracted the beta slope using *lm.beta*[42].
463
464 *16S and ITS bioinformatics*
465 In total, Illumina paired end sequencing of 120 samples (60 16S and 60 ITS amplicons) and 2 negative
466 controls yielded 4 261 236 raw sequences. The data were processed using the pipeline SEED2 ver.
467 2.1.1b[45]. First, low-quality sequences were discarded (mean quality score < 30). The reads were
468 demultiplexed (no mismatch allowed in the tag sequences) and tag switches (i.e. reads with non-
469 matching tags) were discarded.
470 The ITS2 region was extracted from the fungal amplicons using ITSx ver. 1.0.11[46] and sequences
471 shorter than 20 bp were excluded. This yielded 982 036 sequences which were clustered to OTUs using
472 UPARSE implementation in USEARCH ver. 8.1.1861[47] with 97% similarity threshold (45 480 chimeric
473 sequences were excluded during this step). The most abundant sequences were selected for each of
474 the resulting 10 685 OTUs. These sequences were checked for their identity via BLASTn algorithm
475 against the UNITE database ver. 8.3[48] and non-fungal, no-hit sequences as well as global singletons,
476 doubletons and tripletons were excluded from further analyses leaving 2638 OTUs represented by 840
477 206 reads. Six reads detected in the negative control were subtracted from the corresponding two
478 OTUs across the dataset. The ecological guilds of the fungal OTUs were parsed using the database
479 FungalTraits[49] at genus and sequence levels. All abundant OTUs were assigned with high probability.
480 Primers were cut from prokaryote reads (1 319 594 reads after demultiplexing) and sequences
481 shorter than 20 bp were excluded. The reads were clustered to OTUs using UPARSE implementation
482 in USEARCH ver. 8.1.1861[47] with 97% similarity threshold (442 826 chimeric sequences were excluded
483 during this step). OTUs with n < 5 were discarded. The most abundant sequences were selected for
484 each of the resulting 6532 OTUs. These sequences were checked for their identity via BLASTn
485 algorithm against the RDP trainset 16[50]. 179 reads detected in the negative control were subtracted
486 from the corresponding OTUs across the dataset. OTUs with non-target and no BLASTn hits were
487 excluded from further analyses leaving 6369 OTUs represented by 841 512 reads.
488

489 *Microbial community analyses*

490 Significant separation between soil origins was tested with PERMANOVA using *adonis* from the vegan
491 package[43] based on centered log ratio (clr) transformed read counts and visualised using PCA with the
492 phyloseq package[51]. Alpha-diversity (Shannon diversity) was calculated based on multiple rarefaction
493 (1000 iterations) using the metagMisc package[52]. The overlap in OTUs between natural grassland and
494 abandoned arable soil for prokaryotes and fungi was calculated using *ps_venn*[53].

495 For each OTU, we calculated a specialisation index (SI)[54]. SI of the *i*th OTU was calculated as the
496 coefficient of variation minus a correction for under-sampling of rare OTUs:

497 $$SI_i = \left(\frac{\sigma_i}{\mu_i}\right) - \sqrt{\frac{K}{N_i}}$$

498 with σ_i being the standard deviation of the reads of the *i*th OTU across all samples, μ_i being the mean
499 of the reads of the *i*th OTU across all samples, K the number of habitat classes (2 soils) and N the total
500 number of reads of the *i*th OTU across all samples. Calculations were based on rarefied read
501 abundances (rarefied to the smallest sample size: 4517 and 1618 for 16S and ITS, respectively).
502 Average SI of each sample was calculated as the community weighted mean of the SI of all OTUs
503 present in the sample.

504

505 *Microbial network analysis*

506 We constructed co-occurrence networks across the 30 plant communities on each soil origin for both
507 prokaryote (bacteria and archaea) and fungal communities. We first filtered each dataset to exclude
508 rare OTUs with total < 100 reads and OTUs that were present in < 5 samples per soil origin. Co-
509 occurrence networks were then calculated using the SpiecEasi package[55]. In brief, networks were
510 inferred based on clr transformed read counts, neighbourhood selection (MB method) and we
511 selected optimal stability parameters using the StARS selection approach (threshold 0.05, nlambda 20
512 with 999 replications)[56]. We clustered similarly responding OTUs in each network using the Spin-glass
513 algorithm of the igraph package[57–60]. This approach clusters OTUs based on both positive and negative
514 edges as well as their weight. Present and absent edges as well as positive and negative edges were
515 given a similar importance, and unlimited spins (clusters) were provided. Relative read counts were
516 summed per cluster per sample and used in further correlation and structural equation models (SEM).
517 We tested whether networks were significantly more or less densely clustered using a null-model
518 approach. For this, we rewired the original networks edges with preserving the original networks
519 degree distribution using the robin package[61] and calculated the clustering coefficient (modularity) of
520 1000 rewired networks. *P*-values were calculated as the proportion of randomised clustering
521 coefficients smaller than the observed coefficients[3,62].

522 We inferred putative dominant metabolic and functional characteristics of each microbial network
523 cluster. This was done based on similarities, if present, in knowledge of the prokaryote or fungal taxa
524 co-occurring in each network cluster: putative functions were obtained from literature and the
525 FungalTraits database[49], relative habitat specialisation, and enrichment in natural grassland or
526 abandoned arable soil (Fig S8-S9; Table S2-S5). Relative habitat specialisation of network clusters was
527 calculated by taking the community weighted mean of the SI of each sample in each cluster (see
528 Microbial community analysis). Relative habitat generalist clusters were defined as clusters below the
529 community-wide mean SI without overlap of the maximum distribution (75th percentile + 1.5 *
530 interquartile range). Relative habitat specialists were defined as clusters above the community-wide
531 mean SI without overlap of the minimum distribution (25th percentile - 1.5 * interquartile range) (Fig
532 S8)[54].
533 Cluster enrichment in either of the soils was calculated by taking the ln-response ratio of read
534 counts (rarefied) in natural grassland soil divided by read counts in abandoned arable soil for each
535 OTU. Network clusters with response ratios above zero and without overlap of the minimum
536 distribution were defined as enriched in natural grassland soil. Network clusters with response ratios
537 below zero and without overlap of the maximum distribution were defined as enriched in abandoned
538 arable soil (Fig S9).
539
540 *Overall statistics*
541 Analysis of variances were performed using linear mixed-effects models in R version 3.6.1[42]. All models
542 in which 'time' (continuous, scaled) and 'soil origin' were included as an explanatory variable, included
543 'mesocosm' and 'sowing density' as random effects to take repeated measures and initial sowing
544 densities into account. Models were fitted using *lmer* of the lme4 package[63]. Mesocosm position in
545 the garden had negligible effects, in many cases resulted in overfitting of the models and was
546 therefore dropped as a random effect.
547 Correlations between plant, soil chemistry and microbial parameters including network clusters
548 were performed using *lme* of the nlme package[64] and included sowing density as a random factor. For
549 these multiple correlations, p-values were corrected for multiple testing using the Bonferroni
550 correction[65]. In all models, data was ln- or sqrt-transformed when model residuals did not follow a
551 normal distribution. In case of heterogeneity of variances, data weighting per soil origin using *varIdent*
552 from the nlme package was incorporated[66]. Following Tukey HSD post-hoc tests were performed using
553 the package emmeans[67].
554

555 *Structural equation modelling*

556 We hypothesised that plant community parameters in the year of sampling and developmental
557 trajectories in time affected microbial communities with soil chemical changes as a potentially
558 important mediator. We used structural equation models (SEM) which allowed us to separate direct
559 pathways between plant communities and soil microbiota from indirect pathways via soil chemical
560 changes (Fig 4A). We note that the directionality of soil chemistry onto microbial communities is a
561 mathematical necessity to test whether soil chemistry was a significant mediator. Such pathways
562 should however be interpreted as an interaction as both soil microbiota and plant communities
563 influence soil chemistry.

564 All SEMs were fit using piecewiseSEM[68] and *lme* of the nlme package[64] with initial sowing density
565 as a random effect (n = 30). Overall model fit was assessed using direction separation tests (d-sep)
566 based on Fisher's C statistics with models being accepted if $p > 0.1$. We simplified our models using a
567 backward stepwise elimination procedure for which we consecutively removed pathways with the
568 highest p-value[69]. Endogenous variables were allowed to drop from the model in case effects were
569 not significant ($p > 0.05$). The model with the lowest Akaike information criterion (corrected for small
570 sample sizes; AICc) was then selected as the best fit base model (see Supplementary Methods).

571 We created 64 unique SEM models (3 microbial biomass pools natural grassland soil + 3 microbial
572 biomass pools abandoned arable soil + 9 16S clusters natural grassland soil + 10 16S clusters
573 abandoned arable soil + 21 ITS clusters natural grassland soil + 18 ITS clusters abandoned arable soil).
574 We extracted the direction and effect size of each significant pathway of each model ($p < 0.05$) and
575 calculated the contribution of each plant parameter to changes in microbial biomass and microbial co-
576 occurrence clusters. For each significant, direct plant-microbial pathway, we multiplied the path effect
577 size with the relative size of the microbial pool or cluster it was affecting. This multiplication sized the
578 pathway effect to its relative importance to the microbial community as a whole. For indirect
579 pathways, we multiplied the effect size of the plant parameter onto the soil chemical variable with
580 the effect size of the soil chemical variable onto the microbial variable. The obtained effect sizes were
581 scaled to the relative size of the microbial variable they were affecting using community weighted
582 means.

583 To create an overview of all effects, the relative contribution of each plant parameter per microbial
584 variable was summed per group: all combinations of plant parameters in the year of sampling versus
585 past parameters, overall versus compositional parameters, and direct versus indirect pathways (Fig
586 4A). These grouped effect sizes were scaled to the number of potential pathways within each group
587 to allow a direct comparison of the relative contribution of each plant parameter group (Fig 4B).

588

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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; DitZ performed all further microbial, soil and plant data analyses and statistics; DitZ wrote the publication, all others co-commented. All authors contributed critically to the manuscript and gave final approval for publication.

Data and code 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. R scripts are publicly available via: github.com/dintzandt/scripts_stability_and_soil_microbial_networks.

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