Soil multifunctionality is negatively related to microbial community stochasticity in restored grasslands

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AUTHORCONTRIBUTIONS

AR, MS and UG conceived and designed the experiment. AR, MS and MR collected the data. YZ, MR, BF and ZL carried out data analyses. YZ wrote the first draft of the manuscript, and all authors contributed to revisions

DATA ACCESSIBILITY STATEMENT

Should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository (Dryad, Figshare or Hal) and the data DOI will be included at the end of the article.

Abstract

It is generally assumed that there is a relationship between microbial diversity and multiple ecosystem functions. Although it is indisputable that microbial diversity is controlled by stochastic and deterministic ecological assembly processes, the relationship between these processes and soil multifunctionality (SMF) remains less clear. In this study, we examined how different grassland restoration treatments, namely harvest only, topsoil removal and topsoil removal plus propagule addition, affected i) soil bacterial and fungal community stochasticity, ii) SMF, and iii) the relationship between community stochasticity and SMF. Results showed that soil microbial community stochasticity decreased in all the three restoration treatments, while SMF increased. Soil multifunctionality was found to be significantly and negatively correlated with soil microbial community stochasticity. Plant diversity and plant C/N indirectly influenced SMF by regulating the microbial community stochasticity. Our findings provide empirical evidence that when deterministic community assembly processes dominate in soils, then higher microbial functioning is expected.

KEYWORDS

Microbial diversity, plants, topsoil removal, grassland restoration, plant-microbe interaction

INTRODUCTION

Soils simultaneously provide multiple ecological functions and services, e.g., carbon (C) storage, nutrient supply and litter decomposition (Bardgett & Van Der Putten, 2014; Wagg et al., 2014), hereafter referred to as soil multifunctionality (SMF). A large body of research indicates that soil microbial diversity rather than abiotic environmental factors, e.g., climate or topography, is important for SMF (ref). A loss of microbial diversity was shown to reduce SMF (Delgado-Baquerizo et al., 2016; Wagg et al., 2014; Zavaleta et al., 2010), and rare microbial taxa were found to likely be more important for upholding SMF compare to ubiquitous taxa (Chen et al., 2020a; Wei et al., 2019; Xiong et al., 2021). Since soil microbial community assembly processes control microbial diversity, composition and succession patterns, soil microbial community assembly processes likely are also linked to SMF (Wagg et al., 2014; Zheng et al., 2019). However, less is known about the relationship between soil microbial community assembly processes and SMF.

Generally, the soil microbial community assembly is controlled by both deterministic and stochastic processes (Chase, 2010; Chase & Myers, 2011; Guo et al., 2019; Ofiţeru et al., 2010). Deterministic processes are based on the niche theory and ecological selection which suggest that there are specific biotic and abiotic conditions under which species can coexist (Chesson, 2000). Stochastic processes include, in contrast, random birth/death, speciation/extinction, and immigration/emigration (Chave, 2004). Resource limitation, e.g., low nutrient or water availability, largely results in a dominance of deterministic processes (Zhou & Ning, 2017), whereas high

71 nutrient and water supply, or neutral pH as well as random disturbances may lead to a
72 predominance of stochastic processes (Chase, 2010, Chase & Myers, 2011).
73 Deterministic processes tend to enhance the function of microbial communities. For
74 instance, microorganisms enhance the excretion of nitrogen (N) or phosphorus (P)
75 related extracellular enzymes to mineralize N or P from organic material when they are
76 limited by these elements (Gusewell & Freeman, 2005; Yang et al., 2020). Therefore,
77 understanding how soil microbial community assembly processes and SMF are related
78 may provide an insight into the underlying mechanisms that drive SMF.
79 Besides soil microbial diversity, also high plant diversity is generally thought to be
80 important for sustaining high levels of SMF (Berdugo et al., 2017; Sanaei et al., 2021).
81 As aboveground plant diversity shapes belowground community composition by
82 regulating microbial community assembly processes (Liu et al., 2021; Ma et al., 2019),
83 high SMF can, for example, be indirectly mediated by high plant species richness
84 (Sweeney et al., 2021; Wen et al., 2020; Yuan et al., 2020). The impact of plants on soil
85 microbial community assembly processes may be directly related to competition for the
86 same nutrients (e.g., N) (Martínez-García et al., 2015) or via indirect mediation of soil
87 physicochemical properties (Chen et al., 2017). In addition, the quantity and quality of
88 plant and root litter as well as root exudates, i.e., the quantity/quality of C and N
89 returned to the soil, may strongly alter microbial community structure (Adamczyk et al.
90 2021; Chen et al. 2020b). Thus, it would be meaningful to include interactions between
91 plants and soil microorganisms and especially the influence of plant C/N ratios on soil
92 microbial community assembly processes to gain a more in-depth understanding about

how these organisms are linked to SMF.

Here, we took advantage of a 22-year-old restoration experiment in Switzerland to examine the relationship between soil microbial community assembly processes and SMF and how these are linked to plant community properties. The aim of the experiment was to re-connect and enlarge small remnants of oligotrophic semi-natural grasslands, which represent species-rich grassland patches in an otherwise intensively managed species-poor agricultural landscape. Three different restoration methods were tested, namely i) repeated mowing and removing of the harvested plant material, ii) removing of the topsoil (between 10 and 20 cm) and iii) combing topsoil removal with the addition of propagules of plant species from the targeted semi-natural grasslands (Resch et al., 2019). This experimental setup is very well suited to assess soil microbial community assembly processes as we expect that stochastic processes would dominate in microbial communities of the intensively managed agricultural systems (initial systems), while deterministic processes would dominate in the resource limited oligotrophic, semi-natural grasslands, which are our target systems for restoration (Ofiţeru et al., 2010; Zhou et al., 2014). Our restoration treatments are thus expected to support microbial assembly processes that are nested between the two extremes, intensively managed and semi-natural grasslands: community assembly processes in the repeated mowing treatment will be tending more towards the stochastic processes dominating in intensively managed agricultural systems, while after topsoil removal deterministic processes will become more dominant, similar to what is found in the semi-natural grasslands (Dini-Andreote et al., 2015).

Topsoil removal, which is a commonly used method in grassland restoration to mitigate ongoing nutrient-enrichment and the concomitant losses of biodiversity in Europe (Kiehl et al., 2010; Török et al., 2011), is often criticized to be harmful for soil faunal communities and soil functioning due to its massive disturbance. Yet, to date little evidence for this negative impact was provided (Geissen et al., 2013). Hence understanding how soil communities re-assemble and how these processes are related to SMF is an important gap in knowledge to be closed if we want to achieve future conservation and restoration goals.

We hypothesize that (1) SMF will be higher in oligotrophic semi-natural compared to intensively managed agricultural grassland due to higher soil microbial stochasticity and lower microbial diversity in the latter, (2) topsoil removal in agricultural grasslands will decrease soil microbial community stochasticity and enhance diversity in the long-term resulting in SMF similar to that found in oligotrophic grasslands, and (3) plant community properties (e.g., species diversity, shoot C/N) will positively and indirectly affect SMF by controlling soil microbial community stochasticity.

Method

Study area

Our experimental sites were located in the Canton of Zurich, Switzerland, in and around the nature reserve Eigental (47° 27' to 47° 29' N, 8° 37' E). The elevation of the sites ranged from 461 to 507 m, mean annual temperature from 8.9 to 10.6 °C and mean annual precipitation from 910 to 1260 mm (average from 2007 to 2017; MeteoSchweiz, 2018). The soils are classified as calcaric to gleyic Cambisols and Gleysols. The nature

reserve was founded in 1967 to protect small remnants of oligotrophic and species-rich semi-natural grasslands (12 ha overall), enclosed in an intensively managed agricultural landscape. Since these small remnants proved to be too small and too fragmented to conserve high plant species richness, the government of the Canton Zurich decided to re-connect and enlarge these remnants by restoring patches of intensively managed farmland nearby (for detailed information, see Neff et al., 2020; Resch et al., 2019, 2021).

Experimental design

The restoration started in 1995 as an experiment. Three restoration treatments were established in each of eleven patches of farmland: 'Harvest only' (mowing two to three times per year and removal of biomass); 'Topsoil' (removal of 10–20 cm of topsoil); 'Topsoil + Propagules' (topsoil removal combined with the addition of propagules from target plants). In each treatment and patch one permanent plot (5 m x 5 m) was randomly established for a total of 33 plots. Another 11 plots were randomly selected in the adjacent intensively managed farmland, representing the initial conditions ('Initial'), and 11 plots were established in remnants of the targeted species-rich semi-natural grasslands, which represent the 'Target' conditions. In total this led to the establishment of 55 permanent plots (5 treatments × 11 replicates).

Microbial community data

Two soil cores (2.2 cm diameter, 12 cm depth) were randomly collected within a subplot (2 m x 2 m) established at two-meter distance from the permanent plots in mid-July 2017 (see Resch et al., 2021). The two samples were pooled, immediately placed in a

cooler and transported to the laboratory at the Swiss Federal Institute for Forest, Snow and Landscape Research WSL (Birmensdorf, Switzerland) to be stored at -20 °C. The metagenomic DNA was extracted from 8 g sieved soil (2 mm) with the DNeasy PowerMax Soil Kit (Qiagen, Hilden, NRW, Germany) according to the manufacturer`s protocol. Amplification of the V3–V4 region of the prokaryotic small-subunit (16S) and the ribosomal internal transcribed spacer region (ITS2) of eukaryotes was done using primers and PCR conditions as described in Frey et al. (2016). PCRs were run in triplicates, pooled and then paired-end sequenced on the Illumina MiSeq v3 platform (Illumina, San Diego, California, USA) at the Genome Quebec Innovation Centre (Montreal, Quebec, Canada). We used a modified customized pipeline largely based on UPARSE implemented in USEARCH v.9.2 (Edgar, 2013) to conduct quality filtering, clustering into operational taxonomic units (OTUs) and taxonomic assignment (Adamczyk et al., 2019; Frey et al., 2016). High-quality sequences were clustered into OTUs at 97% similarity level after discarding singletons of dereplicated sequences. Taxonomic information was annotated using the most recent versions of SILVA (v.132; Quast et al., 2012) and UNITE (v.8; Nilsson et al., 2019) databases for Prokaryota and Fungi, respectively. Taxonomic assignment with confidence rankings equal or higher than 0.8 were accepted, while rankings below 0.8 were set to unidentified. After rarefying the sequencing depth to the lowest number of sequences for all samples (*rarefy_even_depth* function of 'phyloseq' package; McMurdie & Holmes, 2013), the sequences were classified into 14,025 and 5,800 OTUs for 16S and ITS data, respectively. The Shannon diversity index of both bacteria and fungi were estimated

using the *estimate_richness* function of 'phyloseq' package.

To calculate the relative importance of the stochastic versus deterministic processes of microbial community assembly, we calculated the modified stochasticity ratio (MST) using the 'NST' package (v3.0.6; Ning et al., 2019). We used null model-based approaches for examining community stochasticity, with 50% as the boundary point between more deterministic (< 50%) and more stochastic (> 50%) assemblies.

Soil properties and multifunctionality

For measuring soil chemical and physical properties, we collected three random soil samples with a slide hammer corer (5 cm diameter × 12 cm, AMS Samplers, American Falls ID, USA) in each subplot in mid-June 2017. The three samples were pooled and afterwards divided into two subsamples. The further handling of the two subsamples is described in detail in Resch et al. (2021). Briefly, one subsample was stored at 4°C for measuring ammonium (NH_4^+) and nitrate (NO_3^-) contents, soil potential net nitrogen mineralization (Resch et al., 2021; Risch et al., 2019). Soil respiration (CO_2 fluxes) was assessed during an 8-week incubation period under controlled moisture (60% of field capacity), temperature (20 °C) and light conditions (dark) in the laboratory. We weighed duplicate samples of fresh soil equivalent to 8 g dry soil (24 h at 104 °C) into 50 mL Falcon tubes. The production of CO_2 was measured indirectly every week by the electrical conductivity of the sodium hydroxide solution (calibration by titration of the solution). Carbon dioxide fluxes were calculated as cumulated CO_2 concentrations over the entire sampling time. Soil respiration was corrected for the total incubation time and represented per day values expressed as mg C kg^{-1} soil d^{-1}. The other subsample

was dried for 48 h at 60 °C for measuring soil pH, total and organic C, total N, and cation exchange capacity. For measuring soil physical properties, one additional soil core was collected using a steel cylinder within the core sampler to assure the collection of an undisturbed sample. The cylinder was capped in the field to prevent disturbance. We measured soil field capacity, bulk density, density of fine earth, proportion of skeleton, particle density, total porosity, proportion of fine pores and content of clay, silt and sand (i.e., soil texture) on this sample. We also assessed surface and soil temperature, soil volumetric moisture content, slope inclination and thickness of topsoil horizon for each subplot. For measuring soil microbial biomass carbon (SMC) we used Nanodrop (ND-1000 Spectrometer, Witec AG, Sursee, Switzerland) to determine DNA concentration (ng μl^{-1}) after DNA extraction. We corrected DNA concentration with the dry weight of soil samples. Finally, the corrected DNA concentration was multiplied with the F_{DNA}-factor 6.0 to receive SMC (Joergensen & Emmerling, 2006). We then calculated the soil microbial metabolic quotient (qCO_2) as an index of stress (Bhattacharjya et al., 2021; Singh et al., 2020; Wardle & Ghani, 1995):

$$qCO_2 = \text{soil respiration} / \text{SMC}$$

We then calculated SMF using the averaging approach (Hooper & Vitousek, 1998) as described in detail in Resch et al. (2021). We included soil heterogeneity, soil C storage, water-holding capacity, nutrient retention capacity, and soil potential net N mineralization. For details on how we calculated these five individual functions we refer to Resch et al. (2021). Briefly, soil heterogeneity was calculated using 20 soil properties: soil pH, organic C content, NH_4^+ and NO_3^- concentrations, concentrations

of exchangeable cations [K, Ca, Mg, Na, Mn], bulk density, texture [sand, silt, clay], proportion of skeleton and fine pores, surface and soil temperature, and soil moisture, slope class, thickness of topsoil horizon. Nutrient retention capacity was calculated as the sum of exchangeable cations and protons expresses as $mmol_c$ per 1 kg soil. Soil C storage was corrected for soil depth, stone content, and density of fine earth.

Vegetation data

Plant species were identified on each permanent plot (nomenclature: Lauber & Wagner, 1996) in June 2017 and species cover was visually estimated by the semi-quantitative cover-abundance scale of Braun-Blanquet (1964). We calculated the plant Shannon diversity index based on the matrix of aboveground plant abundance survey data using the R package 'Vegan' (v 2.5-7; Oksanen et al., 2020). Biomass was clipped on a diagonal 2 m x 10 cm rectangle in each permanent plot after plant species identification. Biomass was dried at 60° C for 48 h and weighed. Subsequently, the biomass was ground (Pulverisette 16, Fritsch, Idar-Oberstein, Germany) to pass a 0.5 mm sieve, and shoot C and N were measured by dry combustion using a CN analyzer NC 2500 (CE Instruments, Wigan, United Kingdom).

Network construction

We calculated interaction networks for plants and microorganisms (bacteria, fungi) based on plant species numbers and OTUs using the 'CoNet' software (v 1.1.1. beta; Faust & Raes, 2016) implemented in 'Cytoscape' (v 3.8.2; Shannon et al., 2003). Bacterial or fungal OTU tables were used as first input matrix, and the plant species number table as the second input matrix. Thus, we were able to calculate a bipartite

network of plants and microorganisms. OTUs below a minimum occurrence of five across all the samples were clustered to 'others' when calculate the network. To calculate the interactions between plants and microorganisms, we chose four different methods: Pearson and Spearman rank correlations, Bray-Curtis and Kullback-Leibler nonparametric dissimilarity indices. A maximum of 1000 top-ranking and 1000 bottom-ranking edges were automatically set for each of the four measures. Then the significance of each edge was evaluated from permutation and bootstrap distributions (100 iterations) and Benjamini-Hochberg multiple test correction, and edges with $p < 0.05$ were retained. The output files of the 'CoNet' software were used to calculate network features and for visualization via the 'Gephi' software (v 0.9.2; Bastian et al., 2009). We chose the four following network features: network size (total number of nodes), network connectivity (total number of links), average connectivity (average links per node), ratio of mutual exclusion (ratio of negative to total links). Average connectivity is an indicator for the complexity of the network (Pimm, 1984), high values of ratio of mutual exclusion imply high competition activity between microbes and plants.

Statistical analysis

We tested for treatment differences in stochasticity, diversity and richness of bacteria and fungi as well as SMF using beta regression models (Ferrari & Cribari-Neto, 2004) and likelihood ratio tests (*lrtest* function of the 'lmtest' package, v 0.9-38; Zeileis & Hothorn, 2002). Modified post-hoc pairwise comparison, combining the Bonferroni correction method with false-discovery-rate approach, were applied for multiple testing

(*cld* function of the 'multcomp' package, v 1.4-16; Hothorn et al., 2008). Linear relationships between microbial community stochasticity and SMF as well as between microbial community stochasticity and network features of plant-microbe networks were determined using the *lm* function ('stats' package, v 4.0.5).

We built a structural equation model (SEM) to assess how SMF was influenced by soil microbial community stochasticity, soil microbial alpha-diversity, plant Shannon diversity, plant biomass and plant overall C/N concentrations, and soil chemical and physical properties (soil organic C, soil NO_3^-, soil exchangeable K, soil pH, soil C/N, Supplementary Figure 1). We hypothesize that SMF will be influenced by soil microbial community stochasticity, and soil pH (Delgado-Baquerizo et al., 2016). Soil microbial community stochasticity will be determined by plant community diversity, plant overall C/N, soil nutrients availability (here represented by the concentration of NO_3^- and soil exchangeable K), soil organic C and pH (Jiao & Lu, 2020). Plant Shannon diversity and plant overall C/N will be controlled by soil nutrient availability (Bobbink et al., 2010) and NO_3^- (Sun et al., 2020), respectively (Supplementary Figure 1). Overall goodness of fit for SEMs was evaluated using a Chi-square test ($p > 0.05$ indicates that the observed and expected covariance matrices are not statistically different), the root mean square error of approximation (RMSEA, < 0.08 indicates a good fit) and the goodness-of-fit index (GFI, close to 1 indicates perfect model; Rosseel, 2012). We simplified the *a priori* model by dropping non-significant paths to improve the GFI score while reducing the RMSEA values. The SEM analyses were performed with the 'lavaan' package (v 0.6-8; Rosseel, 2012) using the maximum likelihood estimation method.

All the statistical analyses were conducted in R version 4.0.4 (R Core Team 2021).

RESULTS

The estimated community stochasticity of soil bacteria varied significantly, but somewhat random, between the treatments with mean values of 55% to 72% (Fig. 1a). Against our expectations, stochasticity of 'Initial' did neither differ from the three restoration treatments nor from 'Target'. 'Harvest only', did, in contrast, significantly differ from 'Topsoil + Propagules' and 'Target'. For soil fungi we found the highest percentage of stochasticity community assembly in 'Initial' (76%; Fig. 1b), which corresponds to our hypothesis I. This value was significantly different from all the restored treatments as well as 'Target' (Fig. 1b). In addition, stochasticity was significantly higher in 'Harvest only' compared to 'Topsoil', 'Topsoil + Propagules' and 'Target' (Fig. 1b). Interestingly, and against our expectations, Shannon diversity of the bacterial communities was not affected by restoration as we found no differences between the three treatments and 'Initial'. A significantly lower bacterial Shannon diversity was observed in 'Target' (Fig. 1c). No differences at all were detected between 'Initial', 'Target' and the three treatments for fungal Shannon diversity (Fig 1d).

To verify whether the microbial community received external stress at a lower degree of stochasticity, we calculated qCO_2. qCO_2 was significantly higher in 'Topsoil + Propagules' and 'Target' compared to 'Initial' (Supplementary Fig. 2a). No significant differences were found between 'Topsoil', 'Topsoil + Propagules', 'Target' and 'Harvest only', even though qCO_2 was relatively higher in 'Topsoil', 'Topsoil + Propagules' and 'Target' compared to 'Harvest only' (Supplementary Fig. 2a).

313 The ‘Target’ plots had the highest SMF (Fig. 2). In addition, SMF was significantly
314 higher in all the three restoration treatments compared to ‘Initial’, but significantly
315 lower than in ‘Target’. No significant differences in SMF were observed among the
316 three restoration treatments.

317 Soil bacterial ($R^2 = 0.06$, $p = 0.04$) and fungal ($R^2 = 0.19$, $p < 0.001$) community
318 stochasticity were both negatively related to SMF (Fig 3a, b), which corresponds to our
319 hypothesis II. However, we detected no relationships between SMF and microbial
320 diversity (Fig 3c, d), but the qCO_2 was positively related to SMF (Supplementary Fig.
321 2b)

322 We assessed interactions between plant and bacterial/fungal communities to explore
323 whether these interactions are important for regulating microbial community assembly
324 processes. The networks showed distinct differences in their structure and topology, but
325 were similar for plants-bacteria and plants-fungi (Fig 4, Table S1). Generally, the
326 networks of ‘Initial’ and ‘Harvest only’ were much simpler than the ones of ‘Topsoil’,
327 ‘Topsoil + Propagules’ and ‘Target’ (Fig 4, Table S1).

328 Bacterial community stochasticity was significantly and negatively related to network
329 size, network connectivity, average connectivity, and the ratio of mutual exclusion of
330 the plant-bacteria networks (Fig. 5a-d). Similarly, negative relationships were observed
331 between fungal community stochasticity and network size, network connectivity,
332 average connectivity, but not between fungal community stochasticity and mutual
333 exclusion (Fig. 5e-h).

334 The SEM showed that soil NO_3^- and exchangeable K were negatively related to plant

Shannon diversity (standardized coefficient: -0.57 and -0.36, respectively), while soil NO_3^- negatively affected plant C/N (-0.64; Fig. 6). Plant Shannon diversity and soil exchangeable K had negative (-0.35) and positive direct effects (+0.30) on fungal community stochasticity, respectively. Soil pH had significantly and positively effects on both bacterial (+0.43) and fungal (0.23) community stochasticity. Soil bacterial and fungal community stochasticity were directly and negatively affected by plant C/N (-0.64 and -0.39, respectively). Soil fungal community stochasticity had a significant direct negative effect (-0.31) and soil pH a direct positive effect on SMF (+0.47), while soil bacterial community stochasticity had a marginal direct negative effect on SMF (-0.25, $p = 0.080$). Overall, the SEM explained 33 % of the variance in SMF.

DISCUSSION

We found that fungal community assembly processes were more stochastic in intensively managed grassland ('Initial') where SMF was lower compared to nutrient-poor semi-natural grasslands ('Target'). The three restoration treatments showed values between the two extremes 'Initial' and 'Target'. In contrast to our expectations, we did not find large differences in bacterial stochasticity across treatments. Yet, we detected a strong negative relationship between bacterial stochasticity and SMF.

As suggested by previous studies, highly productive conditions as in our 'Initial' grasslands generally lead to a predominance of stochastic community assembly processes (Chase, 2010; Chase & Myers, 2011) compared to more deterministic processes under resource limited conditions for microbes such as found in our 'Target' grasslands (Zhou & Ning, 2017). A high community stochasticity implies that microbes

357 do not experience much environmental stress, while deterministic processes are more
358 generally found when microbes are experiencing stress. Accordingly, we found high
359 qCO_2, which indicates environmental stress such as nutrient limitation in our nutrient
360 poor 'Target', 'Topsoil' and 'Topsoil + Propagule' plots. Similarly, high N inputs as
361 found in our 'Initial' plots likely lead to lower soil N mineralization, lower abundance
362 of N related functional genes and lower microbial extracellular enzyme activities and
363 therefore lower SMF (Fierer et al., 2012; Jia et al., 2020; Risch et al., 2020), explaining
364 the negative relationships between bacterial and fungal stochasticity and SMF found in
365 our study.
366 In general, we found stronger treatment effects on fungal than bacterial community
367 assembly processes and a stronger relationship between fungal stochasticity and SMF
368 compared to bacterial stochasticity and SMF. This is consistent with findings by
369 Delgado-Baquerizo et al. (2016) and Luo et al. (2018), who reported stronger positive
370 effects of fungal communities on ecosystem multifunctionality than bacterial
371 communities.
372 Also, and against our expectations, microbial diversity did not differ much between
373 agricultural, restored and semi-natural grasslands and was not related to SMF. This
374 contrasts with several other studies reporting that microbial diversity was the main
375 driver of SMF at both regional and global scales (Delgado-Baquerizo et al., 2020;
376 Delgado-Baquerizo et al., 2016; Fan et al., 2021; Li et al., 2020; Linders et al., 2019;
377 Wagg et al., 2019; Zheng et al., 2019). A likely explanation for our results could be
378 decoupling of the microbial diversity from SMF due to functional redundancy, i.e., that

various microbial taxa in a community support the same common functions (Ayala-Munoz et al., 2021; Chen et al., 2020; Kivlin & Hawkes, 2020; Louca et al., 2018; Rousk et al., 2009; Tian et al., 2020; Van Der Heijden et al., 2008; Zhang et al., 2016).

A growing number of studies found links between aboveground and belowground communities (Shen et al., 2021; Xu et al., 2021). We thus hypothesized that plant community diversity and plant traits (shoot C/N) may play key roles in determining SMF by regulating microbial community stochasticity and network complexity. We found much stronger networks between plants and microbes in the topsoil removal treatments as well as 'Target' compared to 'Harvest only' and 'Initial'. The most likely explanation for these interactions could be that in nutrient poor systems soil microbes compete with plants for nutrients, which then results in strong and complex interaction networks and higher SMF (Nordin et al., 2004; Kuzyakov & Xu, 2013). The stronger relationships between stochasticity and plant-microbe network properties we found for fungal compared to bacterial communities could be related to fungal community has more intimate relationship with plants, and is strongly shaped over time by plants compared to bacterial communities (Guo et al., 2019; Hannula et al., 2019; Heinen et al., 2020)

When exploring our findings across treatments with the structural equation model, we were able to confirm the importance of soil microbial stochasticity for SMF, and the crucial role plants play in shaping soil microbial community stochasticity. The negative influence of plant C/N on soil microbial community stochasticity may be due to higher plant C/N ratio in nutrient limited systems (Zhang et al., 2020), which leads to

competition between plants and microbes for N and leads to a decrease in the stochasticity of the microbial community (Dini-Andreote et al., 2015): higher plant diversity may lead to larger soil heterogeneity due to plant specific differences in root exudation and nutrient uptake (Sun et al., 2016). Yet, in our study, we only found a negative effect of plant diversity on fungal but not bacterial community stochasticity. Yet, it is known that plant-fungi interactions likely are much stronger than the ones between plants and bacteria (Frey et al., 2021; Sun et al., 2017; Vandenkoornhuyse et al., 2003). The positive effects of soil pH on microbial community stochasticity in our study are corroborated by a study of (Tripathi et al., 2018). A decrease of soil pH (range from 7.37 to 4.37 in our study) may result in the decrease of soil microbial community stochasticity by exerting more stringent limits on survival and fitness of soil microbes. Soil pH also had a strong direct effect on SMF, which is consistent with the findings of (Delgado-Baquerizo et al., 2020; Delgado-Baquerizo et al., 2016) and was likely related to changes in soil enzymatic activities (Sinsabaugh et al., 2008), cation sorption capacity (Fernandez et al., 2015), and mineral weathering (Tian & Niu, 2015).

Overall, we found that soil microbial community stochasticity was critically important for maintaining soil functions. Interactions between plants and microbes, such as competition for nutrients, were found to be crucial for regulating microbial community stochasticity. Our results suggest that practices that cause limitation of nutrients, such as topsoil removal, may boost the functioning of microbes due to the decrease of soil microbial community stochasticity.

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REFERENCES

Adamczyk, M., Hagedorn, F., Wipf, S., Donhauser, J., Vittoz, P., Rixen, C. et al. (2019) The Soil Microbiome of GLORIA Mountain Summits in the Swiss Alps. *Frontiers in Microbiology*, 10, 1080.

Adamczyk, M., Ruthi, J. & Frey, B. (2021) Root exudates increase soil respiration and alter microbial community structure in alpine permafrost and active layer soils. *Environmental Microbiology*, 23(4), 2152-2168.

Ayala-Munoz, D., Simister, R.L., Crowe, S.A., Macalady, J.L. & Burgos, W.D. (2021) Functional redundancy imparts process stability to acidic Fe(II)-oxidizing microbial reactors. *Environmental Microbiology*. https://doi.org/10.1111/1462-2920.15259.

Bardgett, R.D. & Van Der Putten, W.H. (2014) Belowground biodiversity and ecosystem functioning. *Nature*, 515(7528), 505-511.

Bastian, M., Heymann, S. & Jacomy, M. (2009) Gephi: An Open Source Software for Exploring and Manipulating Networks. *Proceedings of the International AAAI Conference on Web and Social Media*, 3.

Berdugo, M., Kéfi, S., Soliveres, S. & Maestre, F.T. (2017) Plant spatial patterns identify alternative ecosystem multifunctionality states in global drylands. *Nature Ecology & Evolution*, 1(2), 1-10.

Bhattacharjya, S., Adhikari, T., Sahu, A. & Patra, A.K. (2021) Ecotoxicological effect of TiO_2 nano particles on different soil enzymes and microbial community. *Ecotoxicology*, 30(4), 719-732.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M. et al. (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications*, 20(1), 30-59.

Braun-Blanquet, J. (1964) Pflanzensoziologie, Grundzüge der Vegetationskunde (3rd ed). Wien,

453 Austria: Springer
454 Chase, J.M. (2010) Stochastic Community Assembly Causes Higher Biodiversity in More Productive
455 Environments. *Science*, 328(5984), 1388-1391.
456 Chase, J.M. & Myers, J.A. (2011) Disentangling the importance of ecological niches from stochastic
457 processes across scales. *Philosophical transactions of the Royal Society B: Biological sciences*,
458 366(1576), 2351-2363.
459 Chave, J. (2004) Neutral theory and community ecology. *Ecology Letters*, 7(3), 241-253.
460 Chen, Q.-L., Ding, J., Zhu, D., Hu, H.-W., Delgado-Baquerizo, M., Ma, Y.-B. et al. (2020a) Rare
461 microbial taxa as the major drivers of ecosystem multifunctionality in long-term fertilized soils. *Soil*
462 *Biology and Biochemistry*, 141, 107686.
463 Chen, Y.-L., Xu, T.-L., Veresoglou, S.D., Hu, H.-W., Hao, Z.-P., Hu, Y.-J. et al. (2017) Plant diversity
464 represents the prevalent determinant of soil fungal community structure across temperate grasslands
465 in northern China. *Soil Biology and Biochemistry*, 110, 12-21.
466 Chen, Y.C., Ma, S.Q., Jiang, H.M., Hu, Y. & Lu, X.Y. (2020b) Influences of litter diversity and soil
467 moisture on soil microbial communities in decomposing mixed litter of alpine steppe species.
468 *Geoderma*, 377, 114577.
469 Chen, Y.L., Liu, F.T., Kang, L.Y., Zhang, D.Y., Kou, D., Mao, C. et al. (2021) Large-scale evidence for
470 microbial response and associated carbon release after permafrost thaw. *Global Change Biology*,
471 27(14), 3218-3229..
472 Chesson, P. (2000) Mechanisms of Maintenance of Species Diversity. *Annual Review of Ecology and*
473 *Systematics*, 31(1), 343-366.
474 Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D. et al. (2016)

475 Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*,
476 7(1), 1-8.
477 Delgado-Baquerizo, M., Reich, P.B., Trivedi, C., Eldridge, D.J., Abades, S., Alfaro, F.D. et al. (2020)
478 Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature Ecology &*
479 *Evolution*, 4(2), 210-220.
480 Dini-Andreote, F., Stegen, J.C., van Elsas, J.D. & Salles, J.F. (2015) Disentangling mechanisms that
481 mediate the balance between stochastic and deterministic processes in microbial succession.
482 *Proceedings of the National Academy of Sciences of the United States of America*, 112(11), E1326-
483 E1332.
484 Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature*
485 *Methods*, 10(10), 996-998.
486 Fan, K.K., Delgado-Baquerizo, M., Guo, X.S., Wang, D.Z., Zhu, Y.G. & Chu, H.Y. (2021) Biodiversity
487 of key-stone phylotypes determines crop production in a 4-decade fertilization experiment. *The*
488 *ISME Journal*, 15(2), 550-561.
489 Faust, K. & Raes, J. (2016) CoNet app: inference of biological association networks using Cytoscape.
490 *F1000Res*, 5, 1519.
491 Fernandez, M.A., Soulages, O.E., Acebal, S.G., Rueda, E.H. & Sanchez, R.M.T. (2015) Sorption of Zn(II)
492 and Cu(II) by four Argentinean soils as affected by pH, oxides, organic matter and clay content.
493 *Environmental Earth Sciences*, 74(5), 4201-4214.
494 Ferrari, S. & Cribari-Neto, F. (2004) Beta regression for modelling rates and proportions. *Journal of*
495 *Applied Statistics*, 31(7), 799-815.
496 Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A. & Knight, R. (2012) Comparative

497 metagenomic, phylogenetic and physiological analyses of soil microbial communities across
498 nitrogen gradients. *The ISME Journal*, 6(5), 1007-1017.
499 Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F. et al. (2016) Microbial diversity in
500 European alpine permafrost and active layers. *FEMS Microbiology Ecology*, 92(3), 1-17.
501 Frey, B., Walthert, L., Perez-Mon, C., Stierli, B., Köchli, R., Dharmarajah, A. et al. (2021) Deep soil
502 layers of drought-exposed forests harbor poorly known bacterial and fungal communities. *Frontiers*
503 *in Microbiology*, 12, 1061.
504 Geissen, V., Wang, S., Oostindie, K., Huerta, E., Zwart, K.B., Smit, A. et al. (2013) Effects of topsoil
505 removal as a nature management technique on soil functions. *Catena*, 101, 50-55.
506 Guo, Y., Hou, L., Zhang, Z., Zhang, J., Cheng, J., Wei, G. et al. (2019) Soil microbial diversity during 30
507 years of grassland restoration on the Loess Plateau, China: Tight linkages with plant diversity. *Land*
508 *Degradation & Development*, 30(10), 1172-1182.
509 Gusewell, S. & Freeman, C. (2005) Nutrient limitation and enzyme activities during litter decomposition
510 of nine wetland species in relation to litter N : P ratios. *Functional Ecology*, 19(4), 582-593.
511 Hannula, S.E., Kielak, A.M., Steinauer, K., Huberty, M., Jongen, R., Jonathan, R. et al. (2019) Time after
512 time: temporal variation in the effects of grass and forb species on soil bacterial and fungal
513 communities. *MBio*, 10, e02635-19.
514 Heinen, R., Hannula, S.E., De Long, J.R., Huberty, M., Jongen, R., Kielak, A. et al. (2020) Plant
515 community composition steers grassland vegetation via soil legacy effects. *Ecology Letters*, 23(6),
516 973-982.
517 Hooper, D.U. & Vitousek, P.M. (1998) Effects of plant composition and diversity on nutrient cycling.
518 *Ecological Monographs*, 68(1), 121-149.

519 Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models.
520 *Biometrical Journal: Journal of Mathematical Methods in Biosciences*, 50(3), 346-363.
521 Jia, X., Zhong, Y., Liu, J., Zhu, G., Shangguan, Z. & Yan, W. (2020) Effects of nitrogen enrichment on
522 soil microbial characteristics: From biomass to enzyme activities. *Geoderma*, 366, 114256.
523 Jiao, S. & Lu, Y.H. (2020) Soil pH and temperature regulate assembly processes of abundant and rare
524 bacterial communities in agricultural ecosystems. *Environmental Microbiology*, 22(3), 1052-1065.
525 Joergensen, R.G. & Emmerling, C. (2006) Methods for evaluating human impact on soil microorganisms
526 based on their activity, biomass, and diversity in agricultural soils. *Journal of Plant Nutrition and*
527 *Soil Science*, 169(3), 295-309.
528 Kiehl, K., Kirmer, A., Donath, T.W., Rasran, L. & Hölzel, N. (2010) Species introduction in restoration
529 projects–Evaluation of different techniques for the establishment of semi-natural grasslands in
530 Central and Northwestern Europe. *Basic and Applied Ecology*, 11(4), 285-299.
531 Kivlin, S.N. & Hawkes, C.V. (2020) Spatial and temporal turnover of soil microbial communities is not
532 linked to function in a primary tropical forest. *Ecology*, 101(4), e02985.
533 Kuzyakov, Y. & Xu, X. (2013) Competition between roots and microorganisms for nitrogen: mechanisms
534 and ecological relevance. *New Phytologist*, 198(3), 656-669.
535 Lauber, K., & Wagner, G. (1996) Flora Helvetica. Flora der Schweiz. Bern, Switzerland: Haupt.
536 Li, S.F., Huang, X.B., Lang, X.D., Shen, J.Y., Xu, F.D. & Su, J.R. (2020) Cumulative effects of multiple
537 biodiversity attributes and abiotic factors on ecosystem multifunctionality in the Jinsha River valley
538 of southwestern China. *Forest Ecology and Management*, 472, 118281.
539 Linders, T.E.W., Schaffner, U., Eschen, R., Abebe, A., Choge, S.K., Nigatu, L. et al. (2019) Direct and
540 indirect effects of invasive species: Biodiversity loss is a major mechanism by which an invasive

tree affects ecosystem functioning. *Journal of Ecology*, 107(6), 2660-2672.

Liu, L., Zhu, K., Krause, S.M.B., Li, S.P., Wang, X., Zhang, Z.C. et al. (2021) Changes in assembly processes of soil microbial communities during secondary succession in two subtropical forests. *Soil Biology and Biochemistry*, 154, 108144.

Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O'Connor, M.I. et al. (2018) Function and functional redundancy in microbial systems. *Nature Ecology & Evolution*, 2(6), 936-943.

Luo, G., Rensing, C., Chen, H., Liu, M., Wang, M., Guo, S. et al. (2018) Deciphering the associations between soil microbial diversity and ecosystem multifunctionality driven by long-term fertilization management. *Functional Ecology*, 32(4), 1103-1116.

Ma, H., Zou, W., Yang, J., Hogan, J.A., Xu, H. & Chen, J. (2019) Dominant Tree Species Shape Soil Microbial Community via Regulating Assembly Processes in Planted Subtropical Forests. *Forests*, 10(11), 978.

Martínez-García, L.B., Richardson, S.J., Tylianakis, J.M., Peltzer, D.A. & Dickie, I.A. (2015) Host identity is a dominant driver of mycorrhizal fungal community composition during ecosystem development. *New Phytologist*, 205(4), 1565-1576.

McMurdie, P.J. & Holmes, S. (2013) phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, 8(4), e61217.

Neff, F., Resch, M. C., Marty, A., Rolley, J. D., Schütz, M., Risch, A. C., & Gossner, M. M. (2020) Long-term restoration success of insect herbivore communities in seminatural grasslands: a functional approach. *Ecological Applications*, 30(6), e02133.

Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D. et al. (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel

563 taxonomic classifications. *Nucleic Acids Research*, 47(D1), D259-D264.

564 Ning, D., Deng, Y., Tiedje, J.M. & Zhou, J. (2019) A general framework for quantitatively assessing
565 ecological stochasticity. *Proceedings of the National Academy of Sciences*, 116(34), 16892-16898.

566 Nordin, A., Schmidt, I.K. & Shaver, G.R. (2004) Nitrogen uptake by arctic soil microbes and plants in
567 relation to soil nitrogen supply. *Ecology*, 85(4), 955-962.

568 Ofiţeru, I.D., Lunn, M., Curtis, T.P., Wells, G.F., Criddle, C.S., Francis, C.A. et al. (2010) Combined
569 niche and neutral effects in a microbial wastewater treatment community. *Proceedings of the*
570 *National Academy of Sciences*, 107(35), 15345-15350.

571 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D. et al. (2020) Vegan:
572 community ecology package. Version 2.5-7.

573 Pimm, S.L. (1984) The complexity and stability of ecosystems. *Nature*, 307(5949), 321-326.

574 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P. et al. (2012) The SILVA ribosomal
575 RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*,
576 41(D1), D590-D596.

577 R Core Team. (2021) R: A language and environment for statistical computing. R Foundation for
578 Statistical Computing. https://www.R-project.org/

579 Resch, M.C., Schütz, M., Buchmann, N., Frey, B., Graf, U., van der Putten, W.H. et al. (2021) Evaluating
580 long-term success in grassland restoration: an ecosystem multifunctionality approach. *Ecological*
581 *Applications*, 31(00), e02271.

582 Risch, A.C., Zimmermann, S., Moser, B., Schuetz, M., Hagedorn, F., Firn, J. et al. (2020) Global impacts
583 of fertilization and herbivore removal on soil net nitrogen mineralization are modulated by local
584 climate and soil properties. *Global Change Biology*, 26(12), 7173-7185.

585 Risch, A.C., Zimmermann, S., Ochoa-Hueso, R., Schütz, M., Frey, B., Firn, J.L. et al. (2019) Soil net
586 nitrogen mineralisation across global grasslands. *Nature Communications*, 10(1), 4981.
587 Rosseel, Y. (2012) Lavaan: An R package for structural equation modeling and more. Version 0.5–12
588 (BETA). *Journal of Statistical Software*, 48(2), 1-36.
589 Rousk, J., Brookes, P.C. & Bååth, E. (2009) Contrasting soil pH effects on fungal and bacterial growth
590 suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology*,
591 75(6), 1589-1596.
592 Sanaei, A., Ali, A., Yuan, Z., Liu, S., Lin, F., Fang, S. et al. (2021) Context-dependency of tree species
593 diversity, trait composition and stand structural attributes regulate temperate forest
594 multifunctionality. *Science of The Total Environment*, 757, 143724.
595 Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D. et al. (2003) Cytoscape: A
596 Software Environment for Integrated Models of Biomolecular Interaction Networks. *Genome*
597 *Research*, 13(11), 2498-2504.
598 Shen, C., Wang, J., He, J.-Z., Yu, F. & Ge, Y. (2021) Plant diversity enhanced soil fungal diversity and
599 microbial resistance to plant invasion. *Applied and Environmental Microbiology*, 87(11), e00251-
600 21
601 Singh, A.K., Jiang, X.J., Yang, B., Wu, J.N., Rai, A., Chen, C.F. et al. (2020) Biological indicators
602 affected by land use change, soil resource availability and seasonality in dry tropics. *Ecological*
603 *Indicators*, 115, 106369.
604 Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C. et al. (2008)
605 Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, 11(11), 1252-1264.
606 Sun, H., Terhonen, E., Kovalchuk, A., Tuovila, H., Chen, H., Oghenekaro, A.O. et al. (2016) Dominant

607 tree species and soil type affect the fungal community structure in a boreal peatland forest. *Applied*
608 *and Environmental Microbiology*, 82(9), 2632-2643.
609 Sun, S., Li, S., Avera, B.N., Strahm, B.D. & Badgley, B.D. (2017) Soil bacterial and fungal communities
610 show distinct recovery patterns during forest ecosystem restoration. *Applied and Environmental*
611 *Microbiology*, 83(14), e00966-17.
612 Sun, Y., Wang, C., Chen, H.Y. & Ruan, H. (2020) Responses of C: N stoichiometry in plants, soil, and
613 microorganisms to nitrogen addition. *Plant and Soil*, 456(1), 277-287.
614 Sweeney, C.J., de Vries, F.T., van Dongen, B.E. & Bardgett, R.D. (2021) Root traits explain rhizosphere
615 fungal community composition among temperate grassland plant species. *New Phytologist*, 229(3),
616 1492-1507.
617 Tian, D.S. & Niu, S.L. (2015) A global analysis of soil acidification caused by nitrogen addition.
618 *Environmental Research Letters,* 10(2), 024019.
619 Tian, L., Wang, X.W., Wu, A.K., Fan, Y.H., Friedman, J., Dahlin, A. et al. (2020) Deciphering functional
620 redundancy in the human microbiome. *Nature Communications*, 11(1), 1-11.
621 Török, P., Vida, E., Deák, B., Lengyel, S. & Tóthmérész, B. (2011) Grassland restoration on former
622 croplands in Europe: an assessment of applicability of techniques and costs. *Biodiversity and*
623 *Conservation*, 20(11), 2311-2332.
624 Tripathi, B.M., Stegen, J.C., Kim, M., Dong, K., Adams, J.M. & Lee, Y.K. (2018) Soil pH mediates the
625 balance between stochastic and deterministic assembly of bacteria. *The ISME journal*, 12(4), 1072-
626 1083.
627 Van Der Heijden, M.G.A., Bardgett, R.D. & Van Straalen, N.M. (2008) The unseen majority: soil
628 microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*,

11(3), 296-310.

Vandenkoornhuyse, P., Ridgway, K., Watson, I., Fitter, A. & Young, J. (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology*, 12(11), 3085-3095.

Wagg, C., Bender, S.F., Widmer, F. & Van Der Heijden, M.G. (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, 111(14), 5266-5270.

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E. & van der Heijden, M.G.A. (2019) Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nature Communications*, 10(1), 1-10.

Wardle, D.A. & Ghani, A. (1995) A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. *Soil Biology and Biochemistry*, 27(12), 1601-1610.

Wei, X., Hu, Y., Razavi, B.S., Zhou, J., Shen, J., Nannipieri, P. et al. (2019) Rare taxa of alkaline phosphomonoesterase-harboring microorganisms mediate soil phosphorus mineralization. *Soil Biology and Biochemistry*, 131, 62-70.

Wen, Z., Zheng, H., Zhao, H., Xie, S., Liu, L. & Ouyang, Z. (2020) Land-use intensity indirectly affects soil multifunctionality via a cascade effect of plant diversity on soil bacterial diversity. *Global Ecology and Conservation*, 23, e01061.

Xiong, C., He, J.-Z., Singh, B.K., Zhu, Y.-G., Wang, J.-T., Li, P.-P. et al. (2021) Rare taxa maintain the stability of crop mycobiomes and ecosystem functions. *Environmental Microbiology*, 23(4), 1907-1924.

Xu, Y., Dong, S., Gao, X., Yang, M., Li, S., Shen, H. et al. (2021) Aboveground community composition and soil moisture play determining roles in restoring ecosystem multifunctionality of alpine steppe

651 on Qinghai-Tibetan Plateau. *Agriculture, Ecosystems & Environment*, 305, 107163.
652 Yang, Y., Liang, C., Wang, Y.Q., Cheng, H., An, S.S. & Chang, S.X. (2020) Soil extracellular enzyme
653 stoichiometry reflects the shift from P- to N-limitation of microorganisms with grassland restoration.
654 *Soil Biology and Biochemistry*, 149, 107928.
655 Yuan, Z., Ali, A., Ruiz-Benito, P., Jucker, T., Mori, A.S., Wang, S. et al. (2020) Above-and below-ground
656 biodiversity jointly regulate temperate forest multifunctionality along a local-scale environmental
657 gradient. *Journal of Ecology*, 108(5), 2012-2024.
658 Zavaleta, E.S., Pasari, J.R., Hulvey, K.B. & Tilman, G.D. (2010) Sustaining multiple ecosystem functions
659 in grassland communities requires higher biodiversity. *Proceedings of the National Academy of*
660 *Sciences*, 107(4), 1443-1446.
661 Zeileis, A. & Hothorn, T. (2002). Diagnostic checking in regression relationships. *R news*, 2, 7-10.
662 Zhang, J., He, N., Liu, C., Xu, L., Chen, Z., Li, Y. et al. (2020) Variation and evolution of C:N ratio
663 among different organs enable plants to adapt to N-limited environments. *Global Change Biology*,
664 26(4), 2534-2543.
665 Zhang, Y., Cong, J., Lu, H., Deng, Y., Liu, X., Zhou, J. et al. (2016) Soil bacterial endemism and potential
666 functional redundancy in natural broadleaf forest along a latitudinal gradient. *Scientific Reports*,
667 6(1), 28819.
668 Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Dietrich, M. *et al.* (2019) Soil multifunctionality is
669 affected by the soil environment and by microbial community composition and diversity. *Soil*
670 *Biology and Biochemistry*, 136, 107521.
671 Zhou, J., Deng, Y., Zhang, P., Xue, K., Liang, Y., Van Nostrand, J.D. et al. (2014) Stochasticity, succession,
672 and environmental perturbations in a fluidic ecosystem. *Proceedings of the National Academy of*

673 *Sciences*, 111(9), E836-E845.

674 Zhou, J. & Ning, D. (2017) Stochastic Community Assembly: Does It Matter in Microbial Ecology?

675 *Microbiology and Molecular Biology Reviews*, 81(4), e00002-17

Legends

Fig. 1 Treatment effects on soil bacterial (a, c) and fungal (b, d) community stochasticity (BCS, a and FCS, b) and Shannon Index (c, d). I, Initial; H, Harvest only; Ts, Topsoil; TsP, Topsoil + Propagules; T, Target. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Fig. 2 Treatment effects on soil multifunctionality (SMF). I, Initial; H, Harvest only; Ts, Topsoil; TsP, Topsoil + Propagules; T, Target. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Fig. 3 Linear regressions between soil multifunctionality (SMF) and (a) soil bacterial community stochasticity (BCS), (b) soil fungal community stochasticity (FCS), (c) soil bacterial Shannon diversity and (d) soil fungal Shannon diversity. Adjusted R^2, F and p values from linear regression are shown in each panel.

Fig. 4 Treatment effects on interaction networks between plant (species numbers) and microorganisms (OTUs as relative abundance). The upper five panels are the interaction networks between plants and soil bacteria, the lower five panels are the interaction networks between plants and soil fungi. Modules are shown in different colors. The size of the nodes is proportional to their connectivity to other nodes. Details of network topological attributes are listed in Supplementary Table S1. I, Initial; H, Harvest only; Ts, Topsoil; TsP, Topsoil + Propagules; T, Target.

699 Fig. 5 Relationships between microbial community stochasticity and topological
700 attributes of plant and microorganism interaction networks. Relationships between
701 bacterial community stochasticity (BCS) and network size (a), network connectivity (b),
702 average connectivity (c), and mutual exclusion (d); relationships between fungal
703 community stochasticity (FCS) and network size (e), network connectivity (f), average
704 connectivity (g), mutual exclusion (h) data sets. Adjusted R^2, *F* and *p* values from linear
705 regression are shown in each panel. The large points with different colors represent
706 mean values of bacterial community stochasticity or fungal community stochasticity
707 with the standard error as bars. The grey points represent each replicate plot. I, Initial;
708 H, Harvest only; Ts, Topsoil; TsP, Topsoil + Propagules; T, Target.
709
710 Fig. 6 Structural equation model shows the influences of soil pH, NO_3^-, exchangeable
711 potassium (K), plant C/N, plant Shannon diversity (PSD), soil bacterial community
712 stochasticity (BCS), and fungal community stochasticity (FCS) on soil
713 multifunctionality (SMF). The blue lines refer to significant positive relationships,
714 whereas the red lines refer to significant negative relationships. Arrows represent the
715 directional influence of one variable upon another. Values next to the arrows are
716 standardized coefficients. R^2 represents the proportion of variance explained. $^{\#}$, *, **
717 and *** represent significant at levels $p < 0.1$, $p < 0.01$, $p < 0.01$ and $p < 0.001$.
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719

720 Fig. 1

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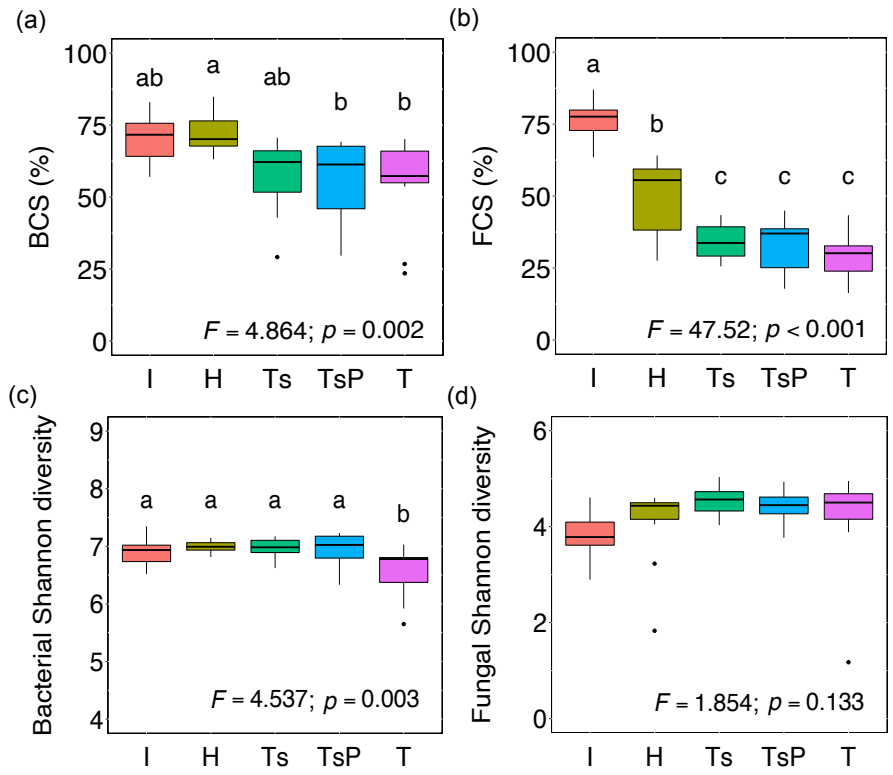

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723 Fig. 2

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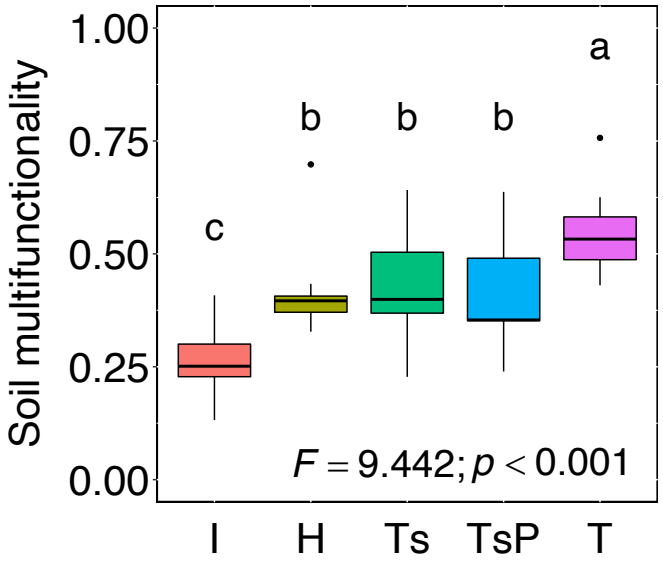

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726 Fig. 3
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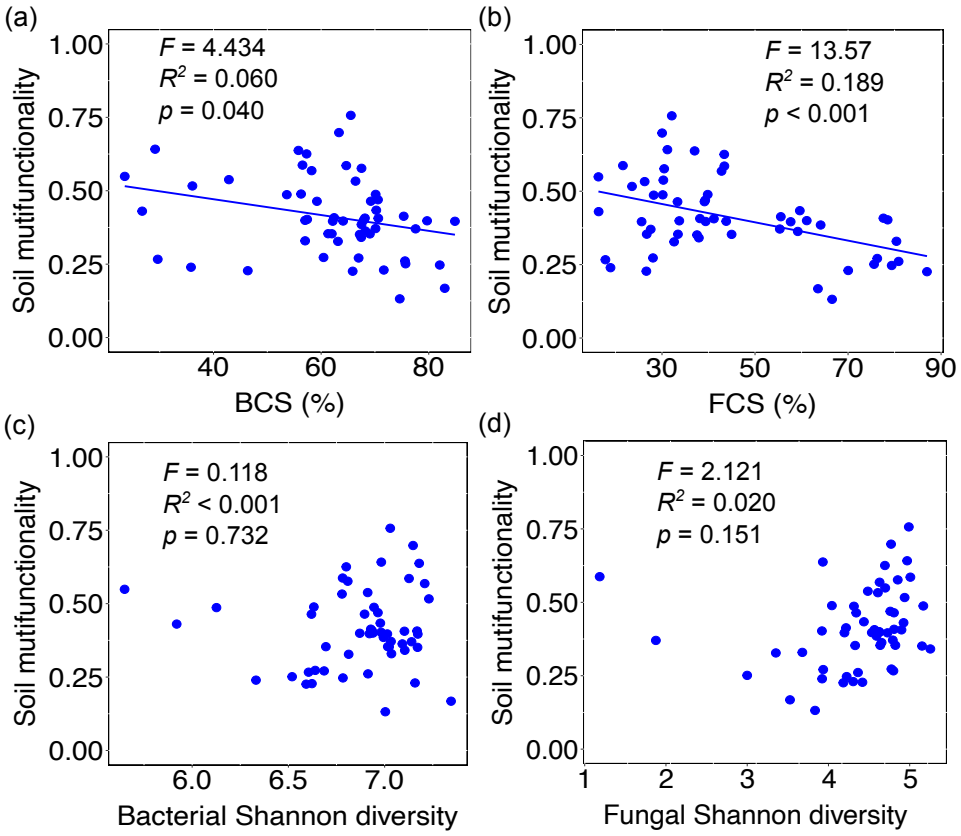

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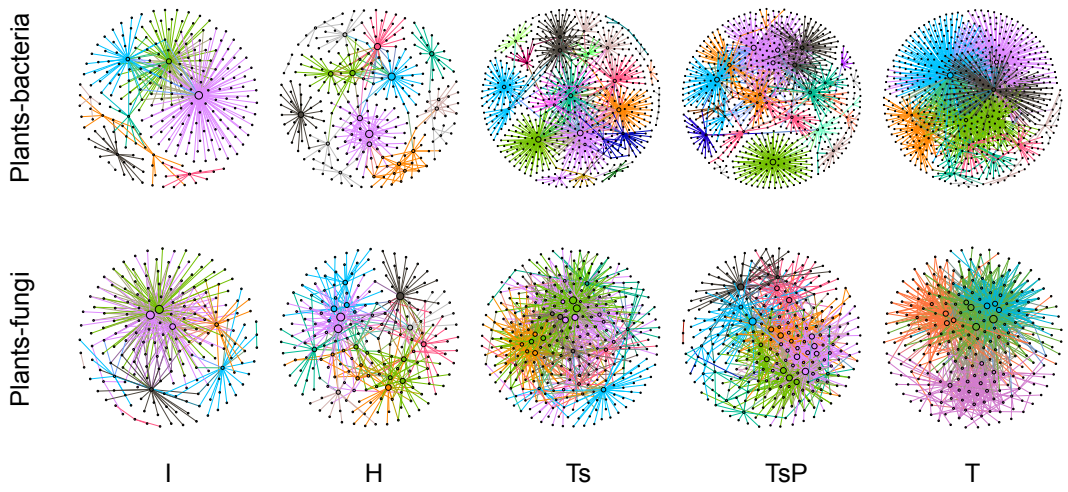
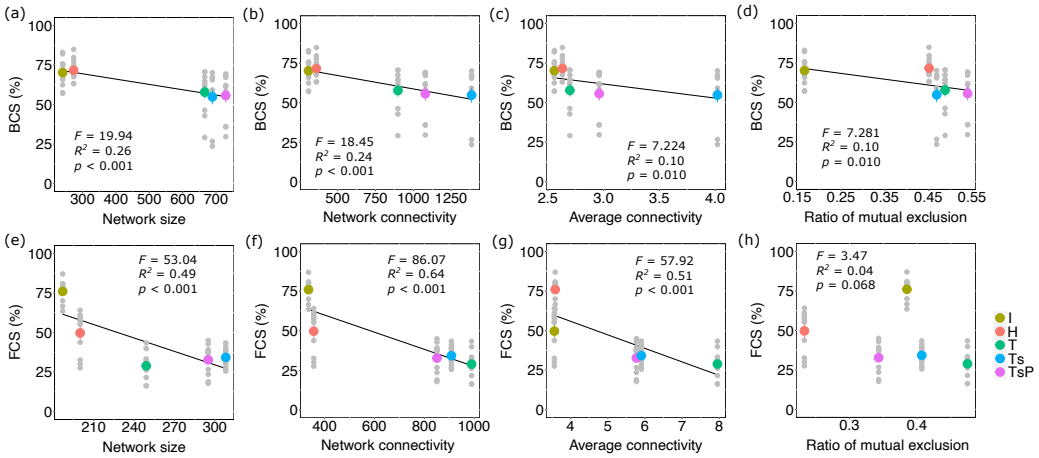
729 Fig.4



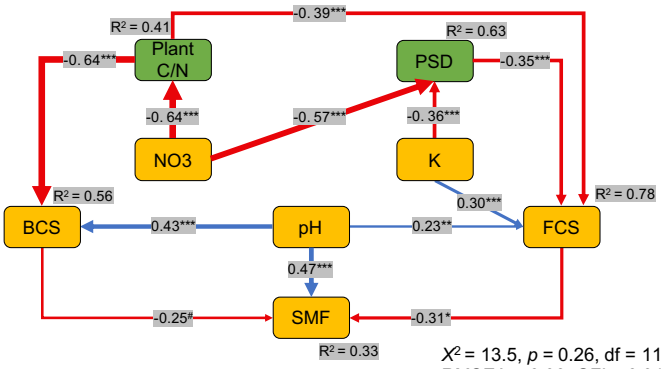
730

731 Fig.5


(a)
BCS (%)
F = 19.94
R² = 0.26
p < 0.001
Network size
(b)
BCS (%)
F = 18.45
R² = 0.24
p < 0.001
Network connectivity
(c)
BCS (%)
F = 7.224
R² = 0.10
p = 0.010
Average connectivity
(d)
BCS (%)
F = 7.281
R² = 0.10
p = 0.010
Ratio of mutual exclusion
(e)
FCS (%)
F = 53.04
R² = 0.49
p < 0.001
Network size
(f)
FCS (%)
F = 86.07
R² = 0.64
p < 0.001
Network connectivity
(g)
FCS (%)
F = 57.92
R² = 0.51
p < 0.001
Average connectivity
(h)
FCS (%)
F = 3.47
R² = 0.04
p = 0.068
Ratio of mutual exclusion
I
H
T
Ts
TsP

732

733 Fig. 6



X^2 = 13.5, p = 0.26, df = 11
RMSEA = 0.06, GFI = 0.94

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