

# Long-term recovery of above-and belowground interactions in restored grasslands after topsoil removal and seed addition

Monika Carol Resch<sup>1</sup>, Martin Schütz<sup>1</sup>, Raúl Ochoa-Hueso<sup>2</sup>, Nina Buchmann<sup>3</sup>, Beat Frey<sup>4</sup>, Ulrich Graf<sup>5</sup>,  
Wim H. van der Putten<sup>6,7</sup>, Stephan Zimmermann<sup>4</sup>, Anita C. Risch<sup>1</sup>

<sup>1</sup>Community Ecology, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, 8903 Birmensdorf, Switzerland

<sup>2</sup>Vitivinicultural and Agri-Food Research Institute, University of Cádiz, 11510 Puerto Real, Spain

<sup>3</sup>Institute of Agricultural Sciences, ETH Zurich, 8092 Zürich, Switzerland

<sup>4</sup>Forest Soils and Biogeochemistry, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, 8903 Birmensdorf, Switzerland

<sup>5</sup>Biodiversity and Conservation Biology, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, 8903 Birmensdorf, Switzerland

<sup>6</sup>Netherlands Institute of Ecology, 6708 Wageningen, The Netherlands

<sup>7</sup>Laboratory of Nematology, Wageningen University, 6700 Wageningen, The Netherlands

## Correspondence:

Monika Carol Resch, WSL Birmensdorf, Switzerland, carol.resch@wsl.ch

**ABSTRACT**

1. Evaluation of restoration activities is indispensable to assess the extent to which targets have been reached. Usually, the main goal of ecological restoration is to restore biodiversity and ecosystem functioning, but validation is often based on a single indicator, which may or may not cope with whole-ecosystem dynamics. Network analyses are, however, powerful tools, allowing to examine both the recovery of various biotic and abiotic properties and the integrated response at community and ecosystem level.
2. We used restoration sites where topsoil was removed from former intensively managed grassland and seeds were added. These sites were between 3 to 32 years old. We assessed how plants, soil biota, soil properties, and correlation-based interactions between biotic communities and their abiotic environment developed over time and compared the results with (i) intensively managed (not restored), and (ii) well-preserved targeted semi-natural grasslands.
3. Plant, nematode, fungal, and prokaryotic diversity and community structures of the restored grasslands revealed clear successional patterns and followed similar trajectories towards targeted semi-natural grasslands. All biotic communities reached targeted diversity levels no later than 18 years post restoration.
4. Ecological networks of intensively managed and short-term (~4 years) restored grasslands were less tightly connected compared to those found in mid- and long-term (~18-30 years) restored and target grasslands. Restoration specifically enhanced interactions among biotic communities, but reduced interactions between biotic communities and their abiotic environment as well as interactions among abiotic properties in the short- and mid-term.
5. *Synthesis and applications*: Overall, our study demonstrated that topsoil removal and seed addition were successful in restoring diverse, tightly coupled and well-connected biotic communities above- and belowground similar to those found in the semi-natural grasslands that were restoration targets. Network analyses proved to be powerful in examining the long-term re-establishment of functionally connected biotic communities in restored ecosystems. Thus, we

provide an approach to holistically assess restoration activities by notably considering the complexity of ecosystems, much in contrast to most traditional approaches.

## KEYWORDS

Ecological networks; grassland restoration; high-intensity restoration method; long-term recovery; restoration success; semi-natural grasslands; soil diversity

## INTRODUCTION

Terrestrial ecosystems are under pressure globally (Vitousek et al., 1997), as almost 40% were converted for agricultural and urban use in the last centuries (Foley et al., 2005). Another 30% have been heavily degraded by unsustainable use of resources, fragmentation, pollution or biological invasion (Millennium Ecosystem Assessment, 2005). Hence, there is an urgent need to restore these degraded, damaged, or destroyed ecosystems (e.g., Gann et al., 2019; Temperton et al., 2019), which recently culminated in the United Nations (UN) proclamation of the “Decade on Ecosystem Restoration” (2021-2030). This action should consolidate global commitments such as the UN Sustainable Development Goals for 2030 (United Nations 2015: Goal 15) to halt and reverse land degradation and the concomitant decline in global biodiversity (Tilman et al., 2001; Kleijn et al., 2009).

In Northwest and Central Europe, land-use change strongly affected highly diverse but low-productive ecosystems, such as semi-natural grasslands (Poschlod et al., 2005). During the past century, these ecosystems were either converted into high-input agricultural areas, or were completely abandoned due to restricted accessibility (e.g., mountainous or inundated areas; Török & Dengler, 2018). In Switzerland, for example, only around 5% of the former semi-natural grasslands remained as small and highly fragmented remnant patches at the beginning of this century (Lachat et al., 2010). Hence, it became necessary to restore, actively extend and re-connect these patches by

70 creating ecological corridors and stepping stones (Dengler et al., 2014). As most of the remnant  
71 patches were embedded in an otherwise intensive agricultural area, conversion of these became a  
72 frequent restoration practice across Europe (see reviews Klimkowska et al., 2007; Kiehl et al., 2010,  
73 Török et al., 2011). Yet, agricultural areas typically exhibited soil nutrient surpluses, homogenized soil  
74 conditions and simplified biotic communities (e.g., McLauchlan, 2006; Gossner et al., 2016; Felipe-  
75 Lucia et al., 2020). To overcome the legacy of intensive agriculture, high-intensity restoration  
76 methods, such as topsoil removal or topsoil removal combined with seed addition, proved to be  
77 most successful in restoring oligo- to mesotrophic semi-natural grasslands (e.g., Klimkowska et al.,  
78 2007; Kiehl & Pfadenhauer, 2007; Kiel et al., 2010; Resch et al., 2019).

79 However, restoration projects using topsoil removal are still rare due to their high costs (Török et  
80 al., 2011), and most of the research conducted in these systems only looked at short-term ( $\leq 10$   
81 years) restoration success. Hence, the number of studies that assessed the long-term success of  
82 topsoil removal is scarce, but slowly increasing. Together these studies showed that plant and insect  
83 communities benefit from topsoil removal and seed addition and successfully develop towards the  
84 targeted reference ecosystems [e.g., Kiehl & Wagner, 2006 (study period: 9 yrs); Wubs et al., 2016 (6  
85 yrs); Resch et al., 2019 (22 yrs); Neff et al., 2020 (22 yrs)]. Belowground recovery lagged, however,  
86 behind the aboveground development and did not reach target levels, not even 20 years after  
87 restoration (Frouz et al., 2009; Geissen et al., 2013; Resch et al., 2021). Thus, it has been argued that  
88 topsoil removal and seed addition create a mismatch between aboveground and belowground  
89 properties (Kardol et al., 2009). Moreover, a delayed belowground recovery may result in poorly re-  
90 established ecological interactions between below- and aboveground properties, which in turn may  
91 entail negative long-term effects on the provisioning of key ecosystem functions (Geissen et al.,  
92 2013). Yet, whether topsoil removal and seed addition are successful in restoring both species-rich  
93 and diverse biotic communities and ecological interactions characteristic for oligotrophic grasslands,  
94 is unknown. Finally, most studies almost exclusively focused on the recovery of plant communities,

while the concurrent recovery of soil biotic communities was widely neglected (but see Kardol et al., 2009; Frouz et al., 2009; Wubs et al., 2016).

In the present study, we analysed diversity and community structure of plants and selected soil biota (nematodes, fungi, prokaryotes) to assess if restoration is successful in re-establishing above- and belowground diversity and ecological interactions. We used eight restoration sites, where topsoil was removed and seeds were added between 3 to 32 years ago, and compared them with intensively managed agricultural areas and targeted semi-natural grasslands. We addressed the following questions: (i) Do diversity and community structure of plants, soil nematodes, fungi and prokaryotes develop towards target after restoration, and if so, how fast? (ii) Do ecological interactions between biotic and abiotic properties develop away from intensively managed and towards targeted semi-natural grasslands? and (iii) Do we find a mismatch between aboveground and belowground recovery, and if so, does it persist in the long-term?

## MATERIAL AND METHODS

### Study area and experimental design

The study was conducted in and around two nature reserves, *Eigental* and *Altläufe der Glatt*, which were located approximately 5 km apart (47°27' to 47°29' N, 8°37' to 8°32' E, 417 to 572 m a.s.l., Canton of Zurich, Switzerland; see Figure S1 and S2, and Table S1 in Supporting Information). Sampling permits for the two nature reserves were issued by the Nature Protection Agency of Canton Zurich and driving permits by the city of Kloten (*Eigental*) and the township of Oberglatt (*Altläufe der Glatt*). Mean annual temperature and precipitation were  $9.8 \pm 0.6$  °C and  $990 \pm 168$  mm, respectively (Kloten climate station 1988-2018; MeteoSchweiz, 2019). The reserves were established in the late 1960ies to protect small remnants of semi-natural grasslands isolated between intensively managed farmland, forests, residential areas and the airport of Zurich (Figure S1). Since the late 1980s, numerous restoration projects were initiated to expand and re-connect these remnants. In

total, 16 hectares of former intensively managed grasslands were restored. To facilitate the re-establishment of targeted grasslands, topsoil removal (upper 10 to 25 cm of the A horizon) was combined with the application of fresh, seed-containing hay and hand-collected seeds from target plant species. Seeding material was always collected from the same nearby semi-dry and semi-wet grassland sites (I. to IV.; Figure S1) located within a radius of 7 to 30 km of the restoration sites (Table S2) and therefore contained native plant species of local and regional provenance. In the rare occasion when non-native species appeared, they were removed manually. The seeding material was generally applied over two consecutive years following topsoil removal. Five years after initial topsoil removal, plant biomass harvest started and still continues once a year in late summer or early autumn (Table S1). The restoration aimed at restoring oligo- to mesotrophic grasslands classified as *Mesobromion* (semi-dry) and *Molinion* (semi-wet) meadows (Delarze et al., 2015).

For this study, we used a space-for-time approach based on eight restoration sites that were between 3 and 32 years old (Figure S1 and S2). We measured recovery and restoration success by comparing the restored grasslands with intensively managed and semi-natural grasslands. Using a space-for-time approach requires high similarities in historical properties of the site, such as soil conditions and management regimes, to assure that temporal processes are appropriately represented by spatial patterns (Walker et al., 2010). This was the case in our study. The restored sites had similar soil conditions (i.e., soil type, structure, water availability) as the targeted semi-natural grasslands, while they shared the same agricultural legacy with intensively managed grasslands, i.e., biomass harvest and fertilization (manure and/or slurry) three to five times a year as well as tillage (Table S1).

We randomly established three 5 m x 5 m (25-m<sup>2</sup>) plots for plant identification, and three 2 m x 2 m (4-m<sup>2</sup>) subplots for soil biotic and abiotic data collection at least 2 m away from the 25-m<sup>2</sup> plots in each restoration site. Sites of similar age were grouped into four age classes: Y.4 (3 & 4 years after restoration), Y.18 (17 & 19 years), Y.24 (23 & 25 years), and Y.30 (27 & 32 years). Six intensively managed (*Initial*) and six semi-natural grassland (*Target*) sites complemented the experimental set-

up, for a total of 36 plots. All plots were sampled under similar conditions, i.e., day of the year, air temperature, soil moisture, and time since last rain event, in June/July 2017 (intensively managed and semi-natural plots) and 2018 (restored plots).

#### **Collection of plants and selected soil biota data**

Plant species cover (in %) was visually estimated in each 25-m<sup>2</sup> plot in mid-June (Braun-Blanquet, 1964; nomenclature: Lauber & Wagner, 1996). We calculated Shannon diversity and assessed plant community structure.

We included soil microbes (fungi, procaryotes) and nematodes in our study as they represent the majority of soil biotic diversity and abundance (Bardgett & van der Putten, 2014), cover various trophic levels of the soil food web (Bongers & Ferris, 1999), and play key roles in soil functioning and ecosystem processes (Bardgett & van der Putten, 2014). In particular, soil nematodes were found to be well suited belowground indicators to evaluate recovery/development after restoration (e.g., Frouz et al. 2008; Kardol et al., 2009; Resch et al., 2019). No ethical approval is needed for the collection of soil nematodes in Switzerland (invertebrate animals). We randomly collected ten undisturbed soil cores (2.2 cm diameter x 12 cm depths) with a soil core sampler (Giddings Machine Company, Windsor, USA) in the 4-m<sup>2</sup> subplots to assess soil nematode and microbial (fungal, prokaryotic) diversities and community structures. For soil nematodes, eight of the soil cores were combined and gently homogenized, placed in coolers and stored at 4 °C and transported to the laboratory (Netherlands Institute of Ecology, NIOO, Wageningen, Netherlands) within three days after collection. Free-living nematodes were extracted from 200 g of fresh soil using Oostenbrink elutriators (Oostenbrink, 1960). After extraction, each sample was divided into three subsamples, two for molecular identification and one to determine nematode abundance (see Resch et al., 2019). For the molecular work, two subsamples were stored in 70% ethanol (final volume 10 mL each) and transported to the laboratory at the Swiss Federal Research Institute WSL (Birmensdorf, Switzerland). Each subsample was reduced to roughly 200 µL by centrifugation and removal of the supernatant. The remaining ethanol was vaporized (65 °C for 3 h). Thereafter, 180 µL ATL buffer solution (Qiagen,

Hilden, Germany) was immediately added and samples were stored at 4 °C until further processing. From these samples, nematode metagenomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, except for the incubation step which was run at 56 °C for 4 h. PCR amplification of the V6-V8 region of the eukaryotic small-subunit (18S) was performed with 7.5 µL of genomic DNA template (ca. 1 ng/µL) in a 25 µL reaction mixture, containing 5 µL PCR reaction buffer, 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.8 µM of each primer (NemF: Sapkota & Nicolaisen, 2015; 18Sr2b: Porazinska et al., 2009), 0.5 µL BSA, and 0.25 µL GoTaq G2 Hot Start Polymerase (Promega Corporation, Madison, USA). Amplification was using an initial DNA denaturation step of 95 °C for 2 min, followed by 35 cycles at 94 °C for 40 sec, 58 °C for 40 sec, 72 °C for 1 min, and a final elongation step at 72 °C for 10 min. Filtering, dereplication, sample inference, chimera identification, and merging of paired-end reads was implemented using the DADA2 pipeline (v.1.12; Callahan et al., 2016) to finally assign amplicon sequence variants (ASVs) as taxonomic units.

We combined and homogenized the remaining two soil cores to assess soil microbes, placed them in coolers (4 °C) and transported them to the laboratory at WSL. Metagenomic DNA was extracted from 8 g sieved soil (2 mm) using the DNeasy PowerMax Soil Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. PCR amplification of the V3-V4 region of the small-subunit (16S) of prokaryotes (i.e., bacteria and archaea) and the ribosomal internal transcribed spacer region (ITS2) of fungi was performed with 1 ng of template DNA using PCR primers and conditions as previously described (Frey et al., 2016). PCRs were run in triplicates, pooled and sent to the Genome Quebec Innovation Centre (Montreal, QC, Canada) for barcoding using the Fluidigm Access Array technology (Fluidigm) and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, USA). Quality filtering, clustering into operational taxonomic units (OTUs, 97% similarity cutoffs) and taxonomic assignment were performed as previously described (Resch et al., 2021).

Taxonomic classification of nematode, prokaryotic and fungal sequences was conducted querying against the most recent versions of PR<sup>2</sup> (v.4.11.1; Guillou et al., 2013), SILVA (v.132; Quast et al.,

2013), and UNITE (v.8; Nilsson et al., 2019) reference sequence databases. Taxonomic assignment cutoffs were set to confidence rankings  $\geq 0.8$  (below ranked as unclassified). Prokaryotic OTUs assigned to mitochondria or chloroplasts as well as OTUs or ASVs assigned to other than *Fungi* or *Nematoda* were manually removed prior to data analysis. The three datasets were filtered to discard singletons and doubletons. Taxonomic abundance matrices were rarefied to the lowest number of sequences per community to achieve parity of the total number of reads between samples (Prokaryotes: 10,929 reads; Fungi: 18,337 reads; Nematodes: 6,662 reads). We calculated Shannon diversity and assessed community structures for soil nematodes, prokaryotes and fungi based on their relative abundances of ASV or OTU at the taxon level.

#### **Collection of soil physical and chemical properties**

We randomly collected one undisturbed soil core (5 cm diameter, 12 cm depth) per 4-m<sup>2</sup> subplot using a steel cylinder that fit into the soil corer. The cylinders were capped to avoid disturbance during transport and used to measure field capacity, rock content, and fine earth density as previously described (Resch et al., 2021). We randomly collected another three soil cores (5 cm diameter, 12 cm depths) in each 4-m<sup>2</sup> subplot to determine soil chemical properties. The cores were pooled, dried at 60 °C for 48 h, and passed through a 2 mm sieve. We measured soil pH (CaCl<sub>2</sub>) on dried samples, total nitrogen (N) and organic carbon (C) concentrations on dried and fine-ground samples ( $\leq 0.5$  mm; for details see Resch et al., 2021). We calculated total N and organic C pools after correcting its concentration for soil depth, rock content and fine earth density.

#### **Statistical analysis**

We used Shannon diversity of plant, nematode, fungal and prokaryotic communities, and soil properties to evaluate if restoration activities succeeded in re-establishing the biotic and abiotic

properties similar to those of the targeted semi-natural grasslands. Effects of time since restoration were assessed using generalized linear mixed effects models with a gamma distribution and an identity link function (Table S3). Normality and homogeneity of Pearson residuals were assessed visually and with Shapiro–Wilk and Levene’s tests. Wald-type Chi-square-Tests were used to test for the overall effect of time since restoration (Anova Type II). Significant differences between the different aged restoration, *Initial* and *Target* plots were identified using least square means, while post hoc p-values were adjusted for multiple comparisons using the Bonferroni correction-method in combination with the false discovery rate approach. To account for the nested structure of the plot arrangement, we used effect of time since restoration as fixed effect and clusters of study areas and restoration sites as random effects (Figure S1, Table S3).

We used principal coordinate analyses (PCoA) based on Bray-Curtis dissimilarity matrices to assess differences in the community structure of plant, nematode, fungi and prokaryote taxa. Dissimilarity matrices of soil biotic communities were based on square root transformed relative abundances per taxonomic unit. To test for significant effect of time since restoration, we conducted permutational multivariate analysis of variance (PerMANOVA) with 10,000 iterations. Values of the first two ordination axes (PCoA.1, PCoA.2) were used to visualize plant, nematode, fungi and prokaryotic community structures. To further visualize differences in community structures, we extracted within- and between-community Bray-Curtis dissimilarity distances and compared dispersions (i.e., standard deviations) of within-community distances of initial/restored grasslands with the respective between-community distances of initial/restored-to-target grasslands.

We used network analyses to evaluate the effect of restoration on ecological interactions between biotic communities and their abiotic environment. These interactions were based on pairwise correlations between biotic and abiotic properties using non-parametric Spearman rank correlations. We used the two ordination axes (PCoA.1, PCoA.2) of plant and soil biotic communities as biotic, and total N pool, organic C pool, pH, bulk density and field capacity as abiotic properties. Interactions between PCoA.1 and PCoA.2 axes within the same biotic community were excluded due

to orthogonality (Risch et al., 2018). We classified ecological interactions at three levels: 1) all possible interactions between biotic communities and abiotic properties, 2) biotic-biotic (i.e., between biotic communities), abiotic-biotic (i.e., between biotic communities and abiotic properties), or abiotic-abiotic (i.e., between abiotic properties) interactions, and 3) interactions related to only plants, nematodes, fungi, or prokaryotic communities and abiotic properties.

Ecosystem coupling was calculated as the average correlation strength between biotic communities and abiotic properties (Risch et al., 2018; Ochoa-Hueso et al. 2021) for the different aged restoration, *Initial* and *Target* grasslands. In total, we considered 74 interactions (24 biotic-biotic, 40 abiotic-biotic, 10 abiotic-abiotic). We assessed overall ecosystem coupling as well as biotic-biotic, abiotic-biotic, and abiotic-abiotic coupling. The average strengths of coupling were then compared against a randomly generated null model derived from the same dataset to test if correlations only happened by chance. Finally, we calculated one-tailed p-values (permutation tests  $n = 999$ ) to test whether properties were significantly and positively coupled in restored, initial and target grassland (Table S4).

We only used significant Spearman's rank correlation coefficients ( $\rho \leq |0.4501|$ ,  $p \leq 0.005$ ; Graham, 2003) to assess strength and structure of the ecological networks. We calculated the number of significant interactions, the summed interaction strength (i.e., connectedness) as well as network connectance (i.e., proportion of significant interactions of all possible interactions; Table 1, Table S5). We used weighted and undirected network graphs based on biotic and abiotic properties to visualize interaction strength and network structure for the different aged restoration, initial and target grasslands. All statistical analyses and graphical outputs were performed in R version 3.6.0 (R Core Team, 2019). A full list of all R packages and functions used can be found in Table S6.

## RESULTS

Restoration had an immediate positive effect on plant diversity, with *H*-values similar to *Target* already 4 years after topsoil removal (Fig. 1a). Restoration had a short-term negative effect on soil nematode diversity, with significantly lower values in *Y.4* compared to *Initial*, *Target* and all other ages of restoration (Fig. 1b). Soil fungal and prokaryotic diversities did not differ between restored, *Initial* or *Target* grasslands at any time (Figs. 1c-d). The structure of all plant and soil biotic communities significantly differed from *Initial* already 4 years after restoration, and after 18 years they could no longer be statistically distinguished from *Target* (Figs. 1e-l). Hence, plant and soil communities were successfully restored in the mid- to long-term.

Soil N and organic C pools were severely reduced by topsoil removal (approx. 60%) in the short-term, but recovered over time, reaching *Target* levels 30 years post restoration (Table S3). Soil pH was slightly higher after restoration, but not significantly different from *Target* (Table S3). Restoration did not have a strong impact on bulk density and field capacity (Table S3).

Restoration changed the overall degree of ecosystem coupling and led to greater coupling within mid- and long-term restored grasslands that was similar to *Target* (Fig. 2a, Table S4). This trend was mainly driven by biotic-biotic coupling, which reached highest values 18- and 24-years after restoration (Fig. 2b). In *Initial* and short-term restored grasslands, biotic communities were not coupled (biotic-biotic coupling not different from null model; Fig. 2b, Table S4). Abiotic-biotic coupling was highest in *Target* and 30 years post restoration, while all other ages of restoration showed abiotic-biotic coupling more similar to *Initial* (Fig. 2c). Only *Initial*, *Y.24* and *Y.30* featured significant abiotic-abiotic coupling (Fig. 2d, Table S4).

Ecological networks strongly differed between *Initial*, restored and *Target* over time (Fig. 3, Table 1, Table S5). Ecological networks were less tightly connected in *Initial* compared to those found in mid- and long-term (*Y.18-30*) restored grasslands and *Target* (Table 1). Yet, restoration had a short-term (*Y.4*) negative effect on ecological networks as the number of interactions, connectedness and connectance were lower than in *Initial* (Fig. 3, Table 1), related to reduced abiotic-abiotic and abiotic-

biotic interactions (Fig. 3, Table 1). Biotic-biotic interactions were, in general, lowest in *Initial*, increased immediately after restoration and even exceeded *Target* values in mid- and long-term restored grasslands (Fig. 3, Table 1, Table S5).

## DISCUSSION

Over time, topsoil removal with seed addition succeeded in re-establishing diverse, tightly coupled and well-connected plant and soil biotic communities that were similar to those found in targeted grasslands. Thus, the disruptive effects of topsoil removal on soil diversity, community structure, and ecological interactions only prevailed in the short-term. Therefore, our study underlines the importance of reducing soil fertility (topsoil removal) to allow restored ecosystems to enter into a new trajectory towards the targeted oligotrophic ecosystem, even at the cost of breaking the existing ecological interactions right after restoration.

We detected that plants and soil biota developed in parallel towards the targeted ecosystems after restoration, but ecological interactions between abiotic and biotic properties were not well re-established shortly after restoration (Y.4). This is similar to earlier findings where a mismatch between the development of plants and soil organisms was found in year 5 after restoration (Kardol et al., 2009). Fully functional connections between plants and soil communities likely only emerge after longer time scales. In fact, diversity and community structures of all biotic communities reached *Target* levels 18 years post restoration. Our study is the first to show that the restoration of semi-natural grasslands using topsoil removal and seed addition benefits both, the primarily targeted plant community as well as soil nematode and microbial communities in the long-term. A timeframe of 10 to 25 years is, however, necessary (Delarze et al., 2016).

We showed that soil abiotic properties, in particular soil N and organic C pools, recovered rather slowly compared to the soil biota, which is in line with results reported from other short- to mid-term studies (Kiehl & Pfadenhauer, 2007; Frouz et al., 2009; Kardol et al., 2009; Geissen et al., 2013). After

the initial reduction in these pools by topsoil removal, it took two decades to restore levels similar to those found in intensively managed grasslands and another decade until *Target* levels were reached. Nevertheless, our results emphasize the positive effects of high-intensity restoration methods to restore important soil functions over time, and thereby revealed their potential contribution to help mitigating future global change effects.

Restoration using topsoil removal to permanently reduce soil nutrient surpluses is discussed quite critically, as it is thought that the soil systems are unable to recover from such severe interventions (Geissen et al., 2013). However, several studies showed that soils and their biotic communities can recover after topsoil removal in the long-term (Frouz et al., 2009; Wubs et al., 2016; Resch et al., 2019). Here we show for the first time that topsoil removal also allows for successful restoration of ecological interactions, which are key for maintaining ecosystem multifunctionality (Morriën et al., 2017; Risch et al., 2018; Resch et al., 2021).

Our findings also confirm that intensive agricultural practices simplify biotic communities, i.e. reduce species numbers, and thus, reduce ecological interactions (Gossner et al., 2016; Felipe-Lucia et al., 2020). Topsoil removal, as expected, destroyed these interactions, but this disruption only persisted in the short-term. In the mid- to long-term, above- and belowground communities including their associated biotic and abiotic interactions swiftly developed towards *Target* grasslands, but still differed from *Target*. The restored systems had for example higher biotic-biotic and abiotic-abiotic but lower abiotic-biotic coupling, resulting in higher overall network strength and connectance. Hence restoration allowed the re-creation of a highly diverse grassland ecosystem with strong ecological interactions, even though some properties might slightly differ from *Target*. Tightly coupled and well-connected ecosystems are more resistant to stress or invasion, and thus more resilient (Morriën et al., 2017; Risch et al., 2018; Felipe-Lucia et al., 2020). This will allow them to better withstand future global change impacts. Hence, our restored grasslands, even though potentially representing an alternative state of the targeted grasslands (Suding et al., 2004; Gann et al., 2019), might be well or even better suited to cope with future challenges than the well-preserved

*Target* grasslands. We are positive that the restored grasslands can not only fulfil their roles as ecological corridors and serve as stepping stones to successfully re-connect remnants of semi-natural grasslands, but also provide important ecological functions and services for the surrounding agricultural areas (Felipe-Lucia et al., 2020; Resch et al., 2021). We would also like to emphasize that future restoration targets should primarily focus on increasing biodiversity and ecosystem functioning in general, independent of whether all species of the target ecosystem will re-establish.

Overall, our study demonstrated that high-intensity restoration methods, such as topsoil removal and seed addition, are successful in converting former intensively managed grasslands into species-rich, tightly coupled and well-connected grassland ecosystems in the long-term. However, the time necessary to reveal the positive effects - high levels of biodiversity and ecosystem functioning - clearly exceed the common evaluation period of restoration projects, which is usually between 3 and 6 years. Thus, we strongly recommend that future monitoring of restoration activities covers several decades and incorporates above- and belowground properties equally to allow for a comprehensive assessment and to capture different “recovery speeds” characteristic for plant and soil communities. Such an approach is not necessarily much more costly compared to the assessment of plant species richness that is typically used in restoration monitoring. Specific combinations of low-cost measures of above- and belowground properties might even be better indicators for restoration success (Resch et al., 2021). Further, our study also indicates that topsoil removal is a successful method that helps to restore intensively used agricultural areas of low ecological value entering into a new trajectory. Given that more tightly coupled and well-connected ecological networks together with higher biodiversity levels and greater soil N and C storage pools are desirable features of sustainable ecosystems, we encourage land managers to use topsoil removal combined with addition of local seeds to achieve their restoration targets.

## **AUTHOR'S CONTRIBUTIONS**

MCR, ACR, MS, BF, SZ, and NB designed the experiment; MCR, ACR, ROH, WHvdP and MS developed the ideas for the manuscript; MCR and UG collected the data; WHvdP, BF and SZ led the lab work; BF led the sequencing analysis; MCR and ROH analysed the data; MCR, ACR and MS wrote the first draft; all authors contributed critically to the drafts and gave final approval for publication.

**CONFLICTS OF INTERESTS**

The authors state they have no conflicts of interests to declare.

**ACKNOWLEDGEMENTS**

We thank Benjamin Fitzpatrick helping with sampling of soil biota and providing statistical advice; the Genetic Diversity Centre (GDC) of the Department of Environmental System Science at ETH Zurich for advice with analysing molecular data; and Roel Wagenaar, Beat Stierli and Roger Köchli for technical laboratory assistance. We also thank Ursina Wiedmer and Pascale Weber for administrative support. This work was supported by the Swiss National Science Foundation (grant number 31003A\_166654).

**DATA AVAILABILITY**

Data available via the EnviDat Repository (Resch et al. 2021): <https://doi.org/10.16904/envidat.252>. Raw sequences of the soil nematode and microbial communities are deposited in the NCBI Sequence Read Archive under the BioProject accession number PRJNA729365.

**REFERENCES**

- 396 Bardgett, R. D., & van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning.  
397 Nature, 515(7528), 505-511. <https://doi.org/10.1038/nature13855>
- 398 Bongers, T., & Ferris, H. (1999). Nematode community structure as a bioindicator in environmental  
399 monitoring. Trends in Ecology & Evolution, 14(6), 224-228. <https://doi.org/10.1016/S0169->  
400 5347(98)01583-3
- 401 Braun-Blanquet, J. (1964). Pflanzensoziologie, Grundzüge der Vegetationskunde (3rd ed). Springer.
- 402 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016).  
403 DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7),  
404 581-583. <https://doi.org/10.1038/nmeth.3869>
- 405 Delarze, R., Gonseth, Y., Eggenberg, S., & Vust, M. (2015). Lebensräume der Schweiz: Ökologie -  
406 Gefährdung - Kennarten (3rd ed.). Ott.
- 407 Delarze, R., Eggenberg, S., Steiger, P., Bergamini, A., Fivaz, F., Gonseth, Y., Guntern, J., Hofer, G.,  
408 Sager, L., & Stucki, P. (2016). Rote Liste der Lebensräume der Schweiz. Aktualisierte Kurzfassung  
409 zum technischen Bericht 2013. Bundesamtes für Umwelt.
- 410 Dengler, J., Janišová, M., Török, P., & Wellstein, C. (2014). Biodiversity of Palaearctic grasslands: a  
411 synthesis. Agriculture, Ecosystems & Environment, 182, 1-14.  
412 <https://doi.org/10.1016/j.agee.2013.12.015>
- 413 Felipe-Lucia, M. R., Soliveres, S., Penone, C., Fischer, M., Ammer, C., Boch, S., ... & Allan, E. (2020).  
414 Land-use intensity alters networks between biodiversity, ecosystem functions, and services.  
415 Proceedings of the National Academy of Sciences, 117(45), 28140-28149.  
416 <https://doi.org/10.1073/pnas.2016210117>
- 417 Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S., Coe, M. T.,  
418 Daily, G. C., Gibbs, H. K., Helkowski, J. H., Holloway, T., Howard, E. A., Kucharik, C. J., Monfreda, C.,

- 419 Patz, J. A., Prentice, I. C., Ramankutty, N., & Snyder, P. K. (2005). Global consequences of land use.  
420 Science, 309(5734), 570-574. <https://doi.org/10.1126/science.1111772>
- 421 Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F., & Hartmann, M. (2016). Microbial  
422 diversity in European alpine permafrost and active layers. FEMS Microbiology Ecology, 92(3),  
423 fiw018. <https://doi.org/10.1093/femsec/fiw018>
- 424 Frouz, J., van Diggelen, R., Pižl, V., Starý, J., Háněl, L., Tajovský, K., & Kalčík, J. (2009). The effect of  
425 topsoil removal in restored heathland on soil fauna, topsoil microstructure, and cellulose  
426 decomposition: implications for ecosystem restoration. Biodiversity and Conservation, 18(14),  
427 3963-3978. <https://doi.org/10.1007/s10531-009-9692-5>
- 428 Gann, G. D., McDonald, T., Walder, B., Aronson, J., Nelson, C. R., Jonson, J., Hallett, J. G., Eisenberg,  
429 C., Guariguata, M. R., Liu, J., Hua, F., Echeverria, C., Gonzales, E., Shaw, N., Decler, K., & Dixon, K.  
430 W. (2019). International principles and standards for the practice of ecological restoration.  
431 Restoration Ecology, 27(S1), S1-S46. <https://doi.org/10.1111/rec.13035>
- 432 Geissen, V., Wang, S., Oostindie, K., Huerta, E., Zwart, K. B., Smit, A., Ritsema, C. J., & Moore, D.  
433 (2013). Effects of topsoil removal as a nature management technique on soil functions. Catena,  
434 101, 50-55. <https://doi.org/10.1016/j.catena.2012.10.002>
- 435 Gossner, M. M., Lewinsohn, T. M., Kahl, T., Grassein, F., Boch, S., Prati, D., ... & Allan, E. (2016). Land-  
436 use intensification causes multitrophic homogenization of grassland communities. Nature,  
437 540(7632), 266-269. <https://doi.org/10.1038/nature20575>
- 438 Graham, M. H. (2003). Confronting multicollinearity in ecological multiple regression. Ecology,  
439 84(11), 2809-2815. <https://doi.org/10.1890/02-3114>
- 440 Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., ... & Christen, R. (2012). The Protist  
441 Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA

- 442 sequences with curated taxonomy. *Nucleic Acids Research*, 41(D1), D597-D604.  
443 <https://doi.org/10.1093/nar/gks1160>
- 444 Kardol, P., Bezemer, T. M., & van der Putten, W. H. (2009). Soil organism and plant introductions in  
445 restoration of species-rich grassland communities. *Restoration Ecology*, 17(2), 258-269.  
446 <https://doi.org/10.1111/j.1526-100X.2007.00351.x>
- 447 Kiehl, K., & Pfadenhauer, J. (2007). Establishment and persistence of target species in newly created  
448 calcareous grasslands on former arable fields. *Plant Ecology*, 189(1), 31-48.  
449 <https://doi.org/10.1007/s11258-006-9164-x>
- 450 Kiehl, K., & Wagner, C. (2006). Effect of hay transfer on long-term establishment of vegetation and  
451 grasshoppers on former arable fields. *Restoration Ecology*, 14(1), 157-166.  
452 <https://doi.org/10.1111/j.1526-100X.2006.00116.x>
- 453 Kiehl, K., Kirmer, A., Donath, T. W., Rasran, L., & Hölzel, N. (2010). Species introduction in restoration  
454 projects—Evaluation of different techniques for the establishment of semi-natural grasslands in  
455 Central and Northwestern Europe. *Basic and Applied Ecology*, 11(4), 285-299.  
456 <https://doi.org/10.1016/j.baae.2009.12.004>
- 457 Kleijn, D., Kohler, F., Báldi, A., Batáry, P., Concepción, E. D., Clough, Y., Díaz, M., Gabriel, D.,  
458 Holzschuh, A., Knop, E., Kovács, A., Marshall, E. J. R., Tschardtke, T., & Verhulst, J. (2009). On the  
459 relationship between farmland biodiversity and land-use intensity in Europe. *Proceedings of the*  
460 *Royal Society B: Biological Sciences*, 276(1658), 903-909. <https://doi.org/10.1098/rspb.2008.1509>
- 461 Klimkowska, A., Van Diggelen, R., Bakker, J. P., & Grootjans, A. P. (2007). Wet meadow restoration in  
462 Western Europe: a quantitative assessment of the effectiveness of several techniques. *Biological*  
463 *Conservation*, 140(3-4), 318-328. <https://doi.org/10.1016/j.biocon.2007.08.024>
- 464 Lachat, T., Blaser, F., Bösch, R., Bonnard, L., Gimmi, U., Grünig, A., Roulier, C., Sirena, G., Stöcklin, J., &  
465 Volkart, G. (2010). Verlust wertvoller Lebensräume. In T. Lachat, D. Pauli, Y. Gonseth, G. Klaus, C.

- 466 Scheidegger, P. Vittoz, & T. Walter (Eds.), Wandel der Biodiversität in der Schweiz seit 1900: Ist die  
467 Tahlsohle erreicht? (pp. 22-63). Bristol-Stiftung Haupt Verlag.
- 468 Lauber, K., & Wagner, G. (1996). Flora Helvetica. Flora der Schweiz. Haupt.
- 469 McLauchlan, K. (2006). The nature and longevity of agricultural impacts on soil carbon and nutrients:  
470 a review. *Ecosystems*, 9(8), 1364-1382. <https://doi.org/10.1007/s10021-005-0135-1>
- 471 MeteoSchweiz. (2019). Klimabulletin Jahr 2018. MeteoSchweiz.
- 472 Millennium Ecosystem Assessment. (2005). *Ecosystems and Human Well Being: Synthesis*. Island  
473 Press.
- 474 Morriën, E., Hannula, S. E., Snoek, L. B., Helmsing, N. R., Zweers, H., De Hollander, M., ... & van der  
475 Putten, W. H. (2017). Soil networks become more connected and take up more carbon as nature  
476 restoration progresses. *Nature Communications*, 8(1), 1-10.  
477 <https://doi.org/10.1038/ncomms14349>
- 478 Neff, F., Resch, M. C., Marty, A., Rolley, J. D., Schütz, M., Risch, A. C., & Gossner, M. M. (2020). Long-  
479 term restoration success of insect herbivore communities in seminatural grasslands: a functional  
480 approach. *Ecological Applications*, 30(6), e02133. <https://doi.org/10.1002/eap.2133>
- 481 Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D.,  
482 Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2019).  
483 The UNITE database for molecular identification of fungi: handling dark taxa and parallel  
484 taxonomic classifications. *Nucleic Acids Research*, 47(D1), D259-D264.  
485 <https://doi.org/10.1093/nar/gky1022>
- 486 Ochoa-Hueso, R., Delgado-Baquerizo, M., Risch, A. C., Schrama, M., Morriën, E., Barmentlo, S. E.,  
487 Geisen, S., Hannula, S. E., Resch, M. C., Snoek, B. L., & van der Putten, W. H. (2021). Ecosystem  
488 coupling: A unifying framework to understand the functioning and recovery of ecosystems. *One*  
489 *Earth*, 4(7), 951-966. <https://doi.org/10.1016/j.oneear.2021.06.011>

- 490 Oostenbrink, M. (1960). Estimating nematode populations by some selected methods. In N. J. Sasser  
491 & W. R. Jenkins (Eds.), *Nematology* (pp. 85–101). University of North Carolina Press.
- 492 Porazinska, D. L., Giblin-Davis, R. M., Faller, L., Farmerie, W., Kanzaki, N., Morris, K., Powers, T. O.,  
493 Tucker, A. E., Sung, W., Thomas, W. K. (2009). Evaluating high-throughput sequencing as a method  
494 for metagenomic analysis of nematode diversity. *Molecular Ecology Resources*, 9(6), 1439-1450.  
495 <https://doi.org/10.1111/j.1755-0998.2009.02611.x>
- 496 Poschlod, P., Bakker, J. P., & Kahmen, S. (2005). Changing land use and its impact on biodiversity.  
497 *Basic and Applied Ecology*, 6(2), 93-98. <https://doi.org/10.1016/j.baae.2004.12.001>
- 498 Quast C., Pruesse E., Yilmaz P., Gerken J., Schweer T., Yarza P., Peplies J., & Glöckner F. O. (2013). The  
499 SILVA ribosomal RNA gene database project: improved data processing and web-based tools.  
500 Opens external link in new window. *Nucleic Acids Research*, 41 (D1), D590-D596.  
501 <https://doi.org/10.1093/nar/gks1219>
- 502 R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for  
503 Statistical Computing, Vienna, Austria.
- 504 Resch, M. C., Schütz, M., Buchmann, N., Frey, B., Graf, U., van der Putten, W. H., Zimmermann, S., &  
505 Risch, A. C. (2021). Evaluating long-term success in grassland restoration - an ecosystem  
506 multifunctionality approach. *Ecological Applications*, 31(3), e02271.  
507 <https://doi.org/10.1002/eap.2271>
- 508 Resch, M. C., Schütz, M., Graf, U., Wagenaar, R., van der Putten, W. H., & Risch, A. C. (2019). Does  
509 topsoil removal in grassland restoration benefit both soil nematode and plant communities?  
510 *Journal of Applied Ecology*, 56(7), 1782-1793. <https://doi.org/10.1111/1365-2664.13400>
- 511 Resch, M. C., Schütz, M., Ochoa-Hueso, R., Buchmann, N., Frey, F., Graf, U., van der Putten, W. H.,  
512 Zimmermann, S., & Risch, A. C. (2021). Data from: Long-term recovery of above-and belowground  
513 interactions in restored grasslands. *EnviDat*. <https://doi.org/10.16904/envidat.252>.

514 Risch, A. C., Ochoa-Hueso, R., van der Putten, W. H., Bump, J. K., Busse, M. D., Frey, B., Gwiazdowicz,  
515 D. J., Page-Dumroese, D. S., Vandegehuchte, M. L., Zimmermann, S., & Schütz, M. (2018). Size-  
516 dependent loss of aboveground animals differentially affects grassland ecosystem coupling and  
517 functions. *Nature Communications*, 9(1), 1-11. <https://doi.org/10.1038/s41467-018-06105-4>

518 Sapkota, R., & Nicolaisen, M. (2015). An improved high throughput sequencing method for studying  
519 oomycete communities. *Journal of Microbiological Methods*, 110, 33-39.  
520 <https://doi.org/10.1016/j.mimet.2015.01.013>

521 Suding, K. N., Gross, K. L., & Houseman, G. R. (2004). Alternative states and positive feedbacks in  
522 restoration ecology. *Trends in ecology & evolution*, 19(1), 46-53.  
523 <https://doi.org/10.1016/j.tree.2003.10.005>

524 Temperton, V. M., Buchmann, N., Buisson, E., Durigan, G., Kazmierczak, L., Perring, M. P., Sá  
525 Dechoum, M., Veldman, J. W., & Overbeck, G. E. (2019). Step back from the forest and step up to  
526 the Bonn Challenge: How a broad ecological perspective can promote successful landscape  
527 restoration. *Restoration Ecology*, 27(4), 705-719. <https://doi.org/10.1111/rec.12989>

528 Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger,  
529 W. H., Simberloff, D., & Swackhamer, D. (2001). Forecasting agriculturally driven global  
530 environmental change. *Science*, 292(5515), 281-284. <https://doi.org/10.1126/science.1057544>

531 Török, P., & Dengler, J. (2018). Palaearctic grasslands in transition: overarching patterns and future  
532 prospects. In V. R. Squires, J. Dengler, H. Feng, & L. Hua (Eds.), *Grassland of the world: diversity,*  
533 *management and conservation* (pp. 15-26). CRC Press.

534 Török, P., Vida, E., Deák, B., Lengyel, S. & Tóthmérész, B. (2011). Grassland restoration on former  
535 croplands in Europe: an assessment of applicability of techniques and costs. *Biodiversity and*  
536 *Conservation*, 20(11), 2311-2332. <https://doi.org/10.1007/s10531-011-9992-4>

- 537 United Nations. (2015). Sustainable Development Goal 15: Protect, restore and promote sustainable  
538 use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and  
539 reverse land degradation and halt biodiversity loss. In United Nations (Eds.), *Transforming our*  
540 *World: The 2030 Agenda for Sustainable Development* (p. 27).
- 541 Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's  
542 ecosystems. *Science*, 277(5325), 494-499. <https://doi.org/10.1126/science.277.5325.494>
- 543 Walker, L. R., Wardle, D. A., Bardgett, R. D., & Clarkson, B. D. (2010). The use of chronosequences in  
544 studies of ecological succession and soil development. *Journal of Ecology*, 98(4), 725-736.  
545 <https://doi.org/10.1111/j.1365-2745.2010.01664.x>
- 546 Wubs, E. J., van der Putten, W. H., Bosch, M., & Bezemer, T. M. (2016). Soil inoculation steers  
547 restoration of terrestrial ecosystems. *Nature Plants*, 2(8), 1-5.  
548 <https://doi.org/10.1038/nplants.2016.107>

**Table 1:** Effect of time since restoration on ecological interactions overall and interactions among abiotic and biotic properties. Network analyses were based on absolute Spearman rank correlations ( $\rho$ :  $|0.4501|$ ,  $P \leq 0.005$ ; Fig. 2); # = number of significant interactions;  $\Sigma$  = Connectedness (summed interaction strengths); Cnct. = Connectance (proportion of significant interactions of all possible interactions); *Initial* = intensively managed grasslands; *Y.4-30* = restored grasslands; *Target* = semi-natural grasslands.

	All			biotic-biotic			abiotic-biotic			abiotic-abiotic		
	#	$\Sigma$	Cnct.	#	$\Sigma$	Cnct.	#	$\Sigma$	Cnct.	#	$\Sigma$	Cnct.
<i>Initial</i>	39	26.3	52.7	8	5.3	33.3	21	15.5	52.5	6	5.0	60.0
<i>Y.4</i>	32	21.5	43.2	11	6.8	45.8	17	11.6	42.5	4	3.1	40.0
<i>Y.18</i>	48	35.6	64.9	24	18.1	100.0	20	14.5	50.0	4	3.0	40.0
<i>Y.24</i>	47	35.2	63.5	24	19.2	100.0	16	11.2	40.0	7	4.8	70.0
<i>Y.30</i>	48	34.6	64.9	21	15.5	87.5	21	14.5	52.5	6	4.6	60.0
<i>Target</i>	47	32.7	63.5	17	12.7	70.8	25	17.1	62.5	5	2.9	50.0

## FIGURE LEGENDS

**Figure 1:** Effect of time since restoration on biodiversity (a-d), community structure (e-h), and distance to target communities (i-l) for plants, nematodes, fungi and prokaryotes in intensively managed (*Initial*), restored (*Y.4-30*), and semi-natural grasslands (*Target*). H-value = Shannon diversity (mean  $\pm$  SE). Different lower-case letters indicate significant differences between different aged restoration, *Initial* and *Target* plots; Community Structure = Principal coordinate analyses (PCoA); Distance to Target = Bray-Curtis dissimilarity (mean  $\pm$  SD) between initial/restored and *Target* (grey-shaded area) communities.

**Figure 2:** Effect of time since restoration on ecosystem coupling: All interactions (a;  $n = 74$ ), biotic-biotic interactions (b;  $n = 24$ ), abiotic-biotic interactions (c;  $n = 40$ ), and abiotic-abiotic interactions (d;  $n = 10$ ). Red line = null model below which coupling is random; Red dashed lines = maximum/minimum coupling values; Error bars = 95% confidence interval of the mean. Notations above the confidence intervals indicate p-values of permutation-based differences from the null model: ns = not significant, + = 0.05–0.1, \* = 0.05–0.01, \*\* < 0.01 (Table S3). Background points = individual interactions between abiotic-abiotic (yellow), abiotic-biotic (blue), and biotic-biotic (magenta) properties. *Initial* = intensively managed grasslands; *Y.4-30* = restored grasslands; *Target* = semi-natural grasslands.

**Figure 3:** Effect of time since restoration on ecological interactions (Spearman rank correlation) between biotic and abiotic properties: plant, soil nematode, fungal and prokaryotic communities and soil properties. An interaction stands for a significant pairwise correlation between single nodes, while the size of each node is proportional to its number of interactions. The thickness of the interactions is scaled to interaction strength (i.e. rank coefficient). Soil properties and biotic

communities are color-coded. The thickness of the outer circle is scaled to the summed interaction strength within biotic communities or soil properties to illustrate the connectedness within the group (Table S4). *Initial* = intensively managed grasslands; *Y.4-30* = restored grasslands; *Target* = semi-natural grasslands; PCoA.1, PCoA.2 = principal coordinate axis 1 and 2; Total N = total nitrogen pool ( $\text{t ha}^{-1} 12\text{cm}^{-1}$ ); Organic C = organic carbon pool ( $\text{t ha}^{-1} 12\text{cm}^{-1}$ ); pH = soil pH ( $\text{CaCl}_2$ ); BD = bulk density ( $\text{g cm}^{-3}$ ); FC = field capacity (%).





