

## RESEARCH ARTICLE

# Divergent assembly processes? A comparison of the plant and soil microbiome with plant communities in a glacier forefield

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**One sentence summary:** A comparison between bacterial and fungal communities associated with leaves or soil and plant communities suggests different assembly processes shaped by characteristics of the organisms and the habitat.

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## ABSTRACT

Community assembly is a result of dispersal, abiotic and biotic characteristics of the habitat as well as stochasticity. A direct comparison between the assembly of microbial and ‘macrobial’ organisms is hampered by the sampling of these communities in different studies, at different sites or on different scales. In a glacier forefield in the Austrian Alps, we recorded the soil and plant microbiome (bacteria and fungi) and plants that occurred in the same landscape and in close proximity in the same plots. We tested five predictions deduced from assembly processes and revealed deviating patterns of assembly in these community types. In short, microbes appeared to be less dispersal limited than plants and soil microbes, and plants strongly responded to abiotic factors whereas the leaf microbiome was plant species specific and well buffered from environmental conditions. The observed differences in community assembly processes may be attributed to the organisms’ dispersal abilities, the exposure of the habitats to airborne propagules and habitat characteristics. The finding that assembly is conditional to the characteristics of the organisms, the habitat and the spatial scale under consideration is thus central for our understanding about the establishment and the maintenance of biodiversity.

**Keywords:** bacteria; dispersal; environmental filter; fungi; glacier forefield; interaction filter

## INTRODUCTION

Species are heterogeneously distributed at global, regional and local scales. Observed distributions are attributed to the species’ evolutionary history, dispersal abilities, adaptations to

the environment and interactions with other organisms as well as to drift as a stochastic element (Vellend 2010). Species that share these characteristics and those that do not exclude each other may co-occur more frequently than expected by chance

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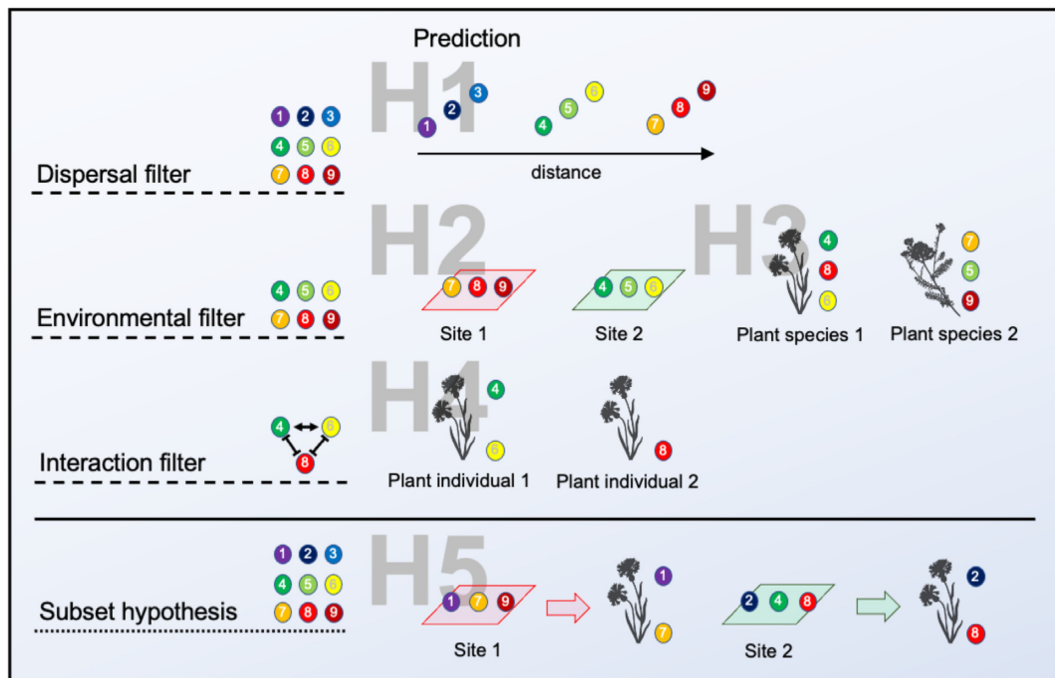
and are thus often part of the same community (Götzenberger *et al.* 2012). Depending on the scale and the organisms under consideration, dispersal filters, environmental filters and/or interaction filters are the dominant processes explaining the composition and diversity of local plant or animal communities (Vellend 2010; Vilmi *et al.* 2021), which may be modulated by evolutionary and metacommunity dynamics (Mittelbach and Schemske 2015). This line of research aiming at identifying the mechanisms underlying species co-occurrence and local diversity is central to ecological theory and nature conservation (HilleRisLambers *et al.* 2012; Kraft *et al.* 2015).

The increasing availability of data on bacterial and fungal communities has fueled the interest in microbial community assembly (Nemergut *et al.* 2013). While the major processes in microbial community assembly are in principle the same as in 'macrobial' communities, striking differences between microorganisms and plant and animals may hamper a direct transfer of concepts and conclusions about the establishment and maintenance of diversity. Active dispersal in microbes is rare or restricted to very short distances, for instance during active chemotaxis under ideal conditions (Raina *et al.* 2019). On the other side, passive airborne dispersal may easily lead to intercontinental distributions of microbes because of smaller propagule sizes (Wilkinson *et al.* 2012). High reproduction rates of microbes, high intraspecific genetic diversity, horizontal gene transfer and rapid evolutionary responses to new habitats enable microorganisms to quickly occupy niches and consume resources (Nemergut *et al.* 2013). Furthermore, and maybe most importantly, microbial community assembly occurs on different spatial scales compared with the assembly of plant and animals. Sampling a single leaf or petal means integrating over multiple microbial niches characterized by different availabilities of water, nutrients and plant metabolites (Karamanoli *et al.* 2012; Hayes *et al.* 2021), whereas a one-square-meter plot may be a rather homogeneous niche for plants. Likewise, a soil particle is characterized by a strong gradient of abiotic variables and thus provides various niches (Sexstone *et al.* 1985). Additionally, mutualistic and antagonistic interactions between microorganisms, which are a dominant factor in shaping microbial co-occurrence, occur on very small scales, often restricted to neighboring cells (Cordero and Datta 2016; Dal Co *et al.* 2020). Thus, field sampling protocols of plant and microbe communities address different organizational levels: a square meter represents a plant community of interacting species that occupying a largely uniform niche; a single leaf or soil particle hosts a number of microbial communities featuring separate interaction networks in diverse niches. Therefore, the relative importance of assembly processes may vary between plant, bacteria and fungi communities despite the fact that they colonize the same landscape. This, however, has not been directly compared.

The selection processes by which members of local communities are filtered from the regional species pool are uniformly considered to be mostly determined by dispersal, abiotic conditions (environment) and species interactions (Fig. 1) (de Bello *et al.* 2012; Götzenberger *et al.* 2012; Cadotte and Tucker 2017), which is basically also covered in Vellend's (2010) conceptual synthesis. The dispersal filter assumes variation in the dispersal abilities of the species present in the regional species pool, which leads to different sets of species that reach a given location. One prediction of the dispersal filter hypothesis is that communities are more similar to each other when they are located in close proximity, meaning that their dissimilarity increases with larger distances between the communities

(Fig. 1, H1). Such a pattern was detected in some microbial systems but not in others (Belisle, Peay and Fukami 2012; Donald *et al.* 2020) but the factors explaining these contrasting results remain unknown. Once organisms reached a given habitat, the environmental filter hypothesis states that abiotic conditions determine whether a species is able to survive and reproduce. This hypothesis predicts that habitats with similar abiotic conditions host more similar communities than habitats that differ in abiotic variables (Fig. 1, H2). The environmental filter hypothesis is well supported for a number of microbial systems (Berg and Smalla 2009; Bulgarelli *et al.* 2013). More specifically for plant-associated microbes, the plant phenotype can be regarded as environment for microbial communities leading to the prediction that plant species host specific microbial communities (Fig. 1, H3). This hypothesis has also been verified in numerous studies (Laforest-Lapointe, Messier and Kembel 2016; Gaube, Junker and Keller 2021) suggesting that plant species-specific properties control microbial colonization (Junker and Tholl 2013; Junker and Keller 2015; Boachon *et al.* 2019). Finally, even in suitable environments, resident species may prevent a successful establishment of further species due to competitive or inhibitory effects. Alternatively, species may also facilitate their establishment. Both of these processes are summarized as interaction filter. In the case of antagonistic interactions, the interaction filter hypothesis would predict lower co-occurrences of species than expected by chance; in case of mutualistic interactions higher co-occurrences than expected by chance (Fig. 1, H4). Interactions between microbes can be strong (Cordero and Datta 2016; Dal Co *et al.* 2020), but whether these interactions contribute to community establishment in microbes remains understudied (Nemergut *et al.* 2013). In this study, we test a fifth hypothesis specific to aboveground plant-associated microbial communities, which does not directly address the assembly process of communities, but rather the source for these microbes. It has been suggested that soil is a reservoir for bacteria associated with roots and also aboveground plant organs (Berg and Smalla 2009; Bulgarelli *et al.* 2013; Bai *et al.* 2015). Thus, these findings suggest that leaf-associated microbial communities consist of a subset of the microbes found in the soil plus those specific to aboveground plant parts (Fig. 1, H5). Using data on community composition to infer assembly processes has been criticized (Gilbert and Bennett 2010; Stegen and Hurlbert 2011; Kraft *et al.* 2015; Stegen *et al.* 2015; Cadotte and Tucker 2017; Blanchet, Cazelles and Gravel 2020) and our tests are not meant to identify the dominant assembly process for the communities under consideration. However, our comparative approach considering communities composed of different organisms that colonize different habitats is well suited to reveal fundamental differences between the ecology and assembly of these communities.

Community assembly processes of bacteria and fungi have been studied either focusing on microbes inhabiting soil or associated with leaves (Schmidt *et al.* 2014; Donald *et al.* 2020; Gao *et al.* 2020; Hassani *et al.* 2020). We estimated the importance of five assembly hypotheses (Fig. 1) for bacterial and fungal communities found in soil and on leaves as well as for plant communities in a comparative approach using null models. These five community types were sampled in the same  $n = 140$  plots along a successional gradient in a glacier forefield in the Austrian Alps, which allows a direct comparison of how the different assembly processes affect different communities within the same landscape. Our study reveals detailed insights into the dispersal abilities of microbes and the biotic and abiotic factors shaping bacterial and fungal communities in comparison to plants and thus



**Figure 1.** Hypotheses on the assembly of communities. Each circle (1–9) represents an operational taxonomic unit (OTU) or species. Hypotheses H1, H2 and H4 are adopted from classical macroscopic community ecology: The dispersal filter (H1) selects species from the regional species pool that are able to reach the habitat. Once the species entered a given habitat, the environmental filter (H2) selects those species that are able to establish and reproduce given the local abiotic conditions. Finally, the interactions filter (H4) selects species that are either facilitated or are at least not outcompeted by the resident species, i.e. a community consists of taxa that have the potential to co-occur. Hypotheses H3 and H5 are more specific to aboveground plant-associated bacteria or fungi. Classically, the environmental filter addresses abiotic parameters such as temperature or pH. The plant itself is, however, the habitat for bacteria and fungi that colonize aboveground plant parts (H3). The properties of these habitats are not only plant organ but also plant species specific; thus, plant species identity is a good proxy for the conditions on the plant habitat. The subset hypothesis (H5) is more about the origin of the microbes than on the assembly. It states that the soil is an important pool for microbes associated with aboveground plant organs. Their establishment on aboveground plant organs is again dependent on assembly processes as described above.

contributes to our understanding about the establishment and the maintenance of biodiversity.

## MATERIALS AND METHODS

### Study site

The study was conducted in the long-term ecological research platform Ödenwinkel (Junker et al. 2020), which was established in 2019 in the Hohe Tauern National Park, Austria (Dynamic Ecological Information Management System—site and data set registry: <https://deims.org/activity/fefd07db-2f16-46eb-8883-f10fb-c9d13a3>, last access: March 2021). A total of  $n = 140$  permanent plots were established in the valley of the Ödenwinkelkees glacier. One hundred thirty-five plots are located within the glacier forefield (from the glacier mouth: plots 1–135), which were covered by ice at the latest glacial maximum in the Little Ice Age (around 1850). The remaining plots (plots 136–140) were established in areas outside the glacier forefield. Plots within the glacier forefield were evenly distributed, representing a successional gradient spanning over 1.7 km in length. Plots were defined as squares with an area of 1 m<sup>2</sup> and were all oriented in the same cardinal direction. Further details on the design of the research platform and exact plot positions, as well as on the sampling strategy, can be found in Junker et al. (2020). During field season in 2019, we estimated the abundance of all vascular plant species growing on the plots and installed a temperature logger (MF1921G iButton, Fuchs Elektronik, Weinheim, Germany) 10 cm north of each plot center, at a depth of 3 cm below ground and calculated the mean seasonal temperature that has

been shown to affect plant species composition as well as interactions between plants and other organisms (Ohler, Lechleitner and Junker 2020). The thermo loggers were set to start on 13 August 2019 and were stopped on 9 August 2020 with a total of 2048 measurements recorded over 362 days. Mean seasonal temperature was calculated on the basis of the recordings ranging from 26 June to 16 September representing the period in which the plots were free of permanent snow cover before and after the winter 2019/2020. Exact coordinates of each plot were directly exported from a GPS device and the distance to closest stream was retrieved from a digital elevation model (1 m LiDAR DEM, Land Salzburg; see Junker et al. 2020). In 2020, soil samples were taken and soil nutrients (Ca, P, K, Mg and total N) as well as soil pH were measured on all plots (except for plot 129) by AGROLAB Agrar und Umwelt GmbH (Landshut, Germany).

### Sampling of microbiome

We sampled microorganisms (bacteria and fungi) inhabiting the phyllosphere of the most frequently occurring plant species and the soil of each plot. Sampling was performed within 11 days during the main vegetative period (31 July 2019–10 August 2019). Leaf and soil samples were collected using sterilized forceps (dipped into 70% ethanol and flamed) to avoid contamination. We sampled bacterial and fungal communities in the phyllospheres of three focus plant species on every plot where they occurred: *Oxyria digyna* as representative of early succession, *Trifolium badium* as representative of late succession and *Campanula scheuchzeri* that occurred all along the successional gradient (for detailed information on the selection of the focus plant species,

see Junker et al. 2020). Furthermore, we took three samples of the most frequently found vascular plant species, i.e. species that occurred on 10 or more plots ( $n = 45$  species). In these cases, we took samples on the oldest, the youngest and the intermediate plot where they occurred. For every plant sample, we took 1 to 3 leaves according to different leaf sizes of the species to make sure that the size of the leaf samples was largely consistent among species. Soil microbiome samples were taken as pooled samples from two locations on every plot whenever there was enough soil to proceed. With a bulb-planting device, we took soil cores, from which we took soil samples at 3 cm depth. In plots where it was not possible to take soil cores due to a lack of developed soil, we collected sediment underneath or next to rocks. Collected samples were directly transferred to ZR BashingBead Lysis tubes containing 750  $\mu\text{L}$  of ZymoBIOMICS lysis solution (Zymo-BIOMICS DNA Miniprep Kit; Zymo Research, Irvine, CA). Within 8 h after collection of microbial samples, ZR Bashing-Bead Lysis tubes were sonicated for 7 min to detach microorganisms from the surfaces. In the case of plant leaves, we removed them from tubes next to a flame with sterile forceps after the sonication to decrease the amount of plant DNA in the samples. Subsequently, all microbial samples were shaken using a ball mill. In cases where we were able to fully remove plant tissues from collection tubes and soil samples, tubes were shaken for 9 min with a frequency of  $30.0 \text{ s}^{-1}$ . In some cases, it was not possible to fully remove plant tissues from tubes, and samples were shaken for 5 min at  $20.0 \text{ s}^{-1}$ . Microbial DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit following the manufacturer's instructions. Next-generation amplicon sequencing and microbiome profiling of isolated DNA samples were performed by Eurofins Genomics (Ebersberg, Germany). Eurofins Genomics amplified and Illumina MiSeq sequenced the V3–V4 region of the 16S rRNA gene to identify bacterial OTUs and the ITS2 region for fungal OTUs following the standard procedure 'InView—Microbiome Profiling 3.0 with MiSeq'. Sequences were demultiplexed, the primers were clipped, forward and reverse reads were merged and merged reads were quality filtered. Microbiome analysis was performed by Eurofins Genomics using the company's standard procedure (the following description of analysis is provided by Eurofins Genomics): reads with ambiguous bases ('N') were removed. Chimeric reads were identified and removed based on the *de novo* algorithm of UCHIME (Edgar et al. 2011) as implemented in the VSEARCH package (Rognes et al. 2016). The remaining set of high-quality reads was processed using minimum entropy decomposition (Eren et al. 2013, 2015). Minimum entropy decomposition (MED) provides a computationally efficient means to partition marker gene data sets into OTUs. Each OTU represents a distinct cluster with significant sequence divergence to any other cluster. By employing Shannon entropy, MED uses only the information-rich nucleotide positions across reads and iteratively partitions large data sets while omitting stochastic variation. The MED procedure outperforms classical, identity-based clustering algorithms. Sequences can be partitioned based on relevant single nucleotide differences without being susceptible to random sequencing errors. This allows a decomposition of sequence data sets with a single nucleotide resolution. Furthermore, the MED procedure identifies and filters random 'noise' in the data set, i.e. sequences with a very low abundance ( $<0.02\%$  of the average sample size). To assign taxonomic information to each OTU, DC-MEGABLAST alignments of cluster representative sequences to the sequence database were performed (Reference database: NCBI.nt [Release 2018-07-07]). A most specific taxonomic assignment for each OTU was then transferred from

the set of best-matching reference sequences (lowest common taxonomic unit of all best hits). Hereby, a sequence identity of 70% across at least 80% of the representative sequence was a minimal requirement for considering reference sequences. Further processing of OTUs and taxonomic assignments was performed using the QIIME software package (version 1.9.1, <http://qiime.org/>) (Caporaso et al. 2010). Abundances of bacterial and fungal taxonomic units were normalized using lineage-specific copy numbers of the relevant marker genes to improve estimates (Angly et al. 2014).

### Test of hypotheses on community assembly

To test the hypotheses on community assembly for specific groups of microbes, we generated the following subsets of the data sets on bacterial and fungal communities: bacteria and fungi (i) associated with plants (bacteria:  $n = 308$ ; fungi:  $n = 324$ ), (ii) colonizing the soil (bacteria:  $n = 132$ ; fungi:  $n = 135$ ) and (iii) associated with three focus plant species (*Campanula scheuchzeri* [bacteria:  $n = 94$ ; fungi:  $n = 113$ ], *Oxyria digyna* [bacteria:  $n = 19$ ; fungi:  $n = 26$ ], *Trifolium badium* [bacteria:  $n = 50$ ; fungi:  $n = 23$ ]). In total, we recorded the composition of  $n = 140$  plant communities. In the following, the different subsets are referred to as 'community types'. Prior to the statistical analysis of microbial communities, we performed a cumulative sum scaling (CSS) normalization (R package *metagenomeSeq* v1.28.2) on the count data to account for differences in sequencing depth among samples. Additionally, to compare the assembly processes of bacteria and fungi to those of plants, we also used plant cover as a surrogate of abundance recorded at all of the  $n = 140$  plots. To test the assembly hypotheses, we first performed the statistical analyses as described below using the field data, and additionally for null models generated from the same data. For each subset of the data we generated  $n = 1000$  null models using the function *nullmodel*, *method = 'r2d'* implemented in the R package *bipartite* (Dormann, Fründ and Gruber 2014). This method generates random community tables with fixed row and column sums using the Patefield's algorithm (Patefield 1981). For each hypothesis and data subset, we generated one test statistic for the field data set and  $n = 1000$  test statistics for the null models generated from the field data. As a measure of deviation of the observed result from the null model expectation, we used one-sample Cohen's  $d = (\text{Mean} - \text{Mu}) / \text{Sd}$  with *Mean* and *SD* as the mean value and standard deviation of null model results and *Mu* as observed result. Higher Cohen's  $d$  values indicate stronger effect. As Cohen's  $d$  thus is based on different test statistics with different ranges (see below) the values are not comparable between the hypotheses, but provide a good measure to compare the effects on different groups (bacteria, fungi detected on leaves or soil, plants) within a hypothesis. Significant differences between null models and observed results were indicated if the observed result did not overlap with the 95% confidence interval of results obtained from null models.

### H1: dispersal filter

If dispersal filter determined the composition of communities, we would expect that communities spatially close to each other are more similar to each other in their composition than communities that are separated by larger distances. As spatial distance we used Euclidean distances based on the latitude, longitude and elevation of the plots where we sampled the communities. For the similarity in community composition, we used Bray–Curtis distances based on the CSS abundance of the OTUs

in the case of bacteria and fungi or the abundance of plants. To test for a correlation between the spatial distance and community distance we performed Mantel test based on Pearson's product-moment correlation, the  $r$ -value was used as test statistic. In the analysis using all samples of leaf associated microbes, potential effects of other assembly rules (niche-based processes) may overlay the effect of dispersal limitation. Therefore, we also performed the analysis only within the samples collected from leaves of one of the three focus species, which represent a more uniform habitat.

## H2 and H3: environmental filter

If environmental filters determined the composition of communities, we would expect that communities established on plots characterized by similar environmental parameters would be more similar to each other compared with communities established in different environments. As environmental distance (H2), we used Euclidean distances based on the soil nutrients (N, P, K, Mg), soil pH, distance to closest stream and the mean seasonal temperature. Additionally, we used the plant species composition as environmental parameter for microbes and used Bray–Curtis distances to calculate the distances between plots. For the similarity in community composition, we used Bray–Curtis distances based on the CSS abundance of the OTUs in the case of bacteria and fungi or the abundance of plants. To test for a correlation between the environmental distance and community distance we performed Mantel statistic based on Pearson's product-moment correlation, the  $r$ -value was used as test statistic. Again, for leaf-associated bacteria and fungi, we repeated this analysis using only the samples collected from leaves of one of the three focus species. For leaf associated bacteria and fungi, not necessarily the soil parameters define the environmental niche, but the physical and chemical properties of the leaves. Therefore, to test hypothesis 3, we performed distance-based redundancy analyses using Bray–Curtis distances followed by 'permutation test under reduced model' to test whether bacterial and fungal communities are more similar within than between plant species. The  $F$ -values of permutation test was used as test statistic. We did this for the whole data set comprising all plant species sampled and also for a subset considering only the three focus species that have a meaningful sample size.

## H4: interaction filter

If interaction filters determined the composition of communities, we would expect that OTUs show a higher co-occurrence (higher aggregation) than expected by chance if facilitation between species is the dominant type of interaction; or show a lower co-occurrence (higher segregation) than expected by chance if competition between species is the dominant type of interaction. To test for species aggregation or segregation we used the `cooc.null.model` function implemented in the R package `EcoSimR`. The  $C$ -score was used as metric to evaluate whether co-occurrence patterns are rather aggregated (low  $C$ -scores) or segregated (high  $C$ -scores). For this hypothesis, we only used data sets on bacteria and fungi associated with leaves of the three focus species—bacteria and fungi in soil, and plant communities—to make sure that the organisms share a common habitat where they can interact.

## H5: subset hypothesis

If soil was a major source of plant-associated bacteria and fungi, we would expect that the proportion of leaf-associated OTUs

that are found in both leaf and soil samples is higher when tested using soil samples from the same plot where the leaf was sampled as compared with soil samples from other plots. Thus, for each plant sample, we first calculated the proportional overlap between leaf-associated OTUs and the OTUs detected in the soil of the plot where the plant was sampled. As a second step, we calculated the proportional overlap between leaf-associated OTUs and the OTUs detected in the soil sampled in all other plots. Therefore, here the second step represents the null model. The mean overlap of leaf-associated microbes with soil microbes on the same plot was used as  $\mu$ , and the mean and standard deviation of the mean overlaps of leaf-associated microbes with soil microbes on different plots as  $Mean$  and  $SD$  in the formula to calculate Cohen's  $d$ .

## RESULTS

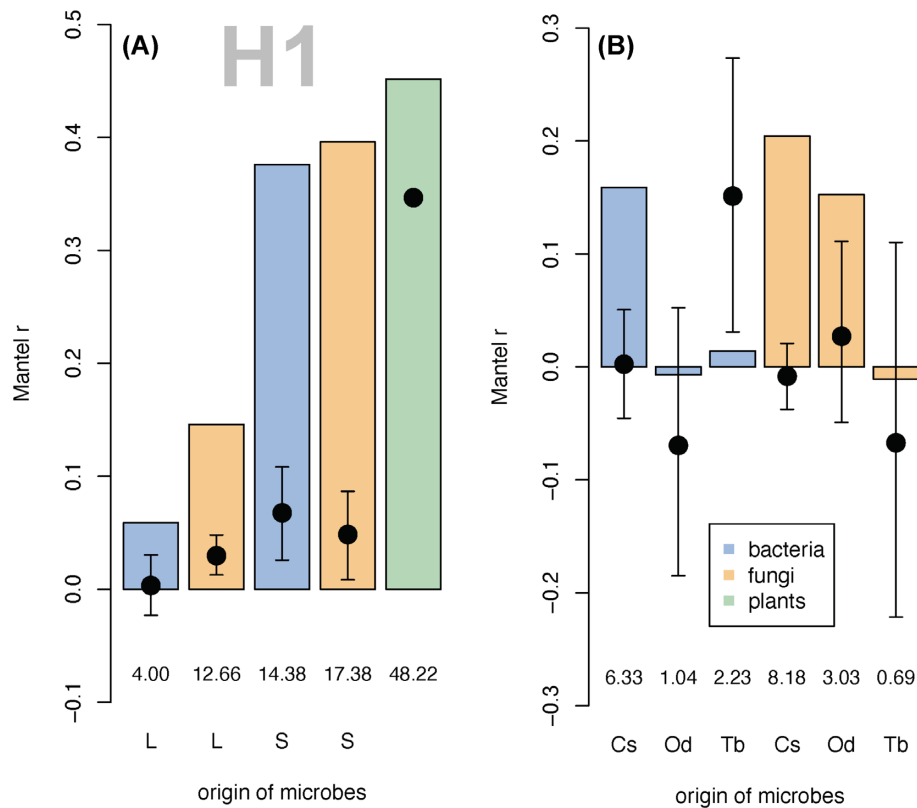
In total, we detected  $n = 10860$  bacterial OTUs associated with plant leaves,  $n = 5221$  bacterial OTUs in soil samples,  $n = 5363$  fungal OTUs associated with plant leaves,  $n = 6014$  fungal OTUs in soil samples and  $n = 108$  plant species. Raw sequences of next-generation 16S rRNA and ITS rRNA gene amplicon sequencing are available at the NCBI Sequence Read Archive (SRA) under the BioProject accession PRJNA701884 and PRJNA701890. The sequencing depth as well as the number of OTUs per samples is given in Supplementary Information 1.

### H1: dispersal filter

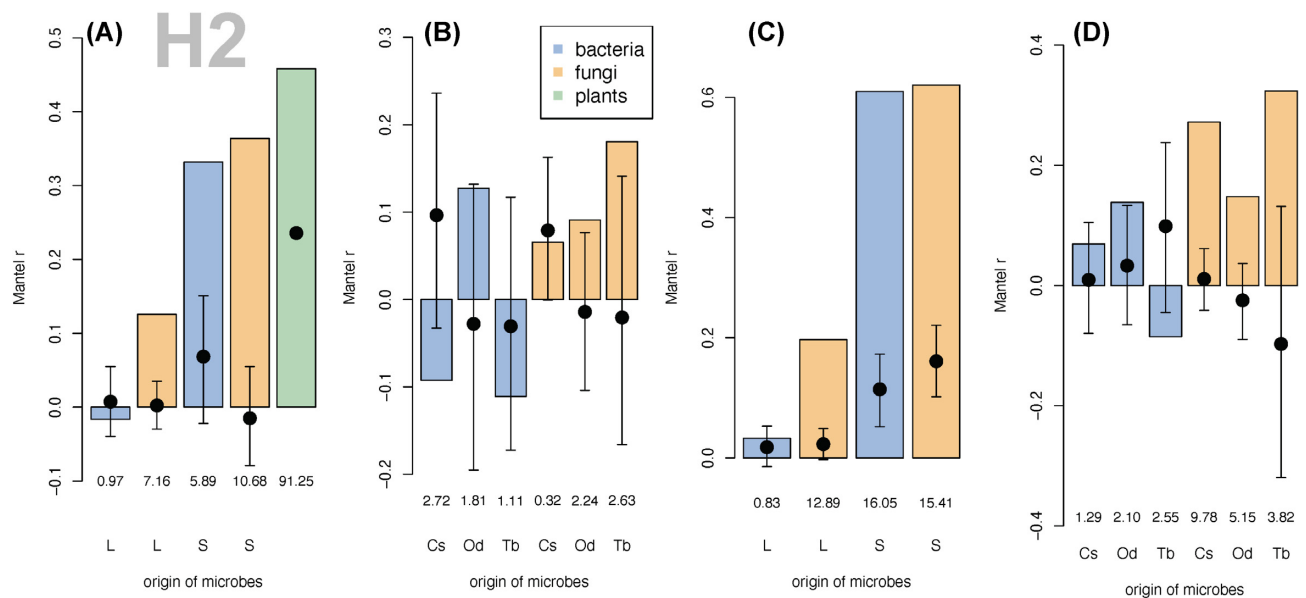
We did find only a weak correlation between the similarity of bacterial and fungal communities associated with leaves and the spatial distance between these communities (Fig. 2A, Supplementary Information 2). In contrast, microbial soil communities as well as plant communities showed a strong correlation between their composition and spatial distance, i.e. communities in close proximity showed a higher similarity in composition than communities separated by larger distances (Fig. 2A, Supplementary Information 2). In all cases the observed Mantel  $r$  was larger than the Mantel  $r$  expected from null models. Microbial communities associated with *Oxyria digyna* and *Trifolium badium* also did not show a strong correlation between compositional similarity and spatial distance (Fig. 2B, Supplementary Information 2). However, communities associated with *C. scheuchzeri* leaves, the plant species with the largest range of distribution within the successional gradient, showed a moderate relationship between compositional similarity and spatial distance (Fig. 2B, Supplementary Information 2) whereas null model expectations were close to zero for Mantel  $r$ .

### H2: environmental filter

To test the environmental filter hypothesis, we used two data sets to characterize the environment of microbes and plants: (i) abiotic factors: soil nutrients (N, P, K, Mg), soil pH, distance to closest stream and the mean seasonal temperature (Fig. 3A and B) and (ii) the biotic environment, which is the composition of plant species growing in each plot (Fig. 3C and D). Similarity in microbial community, compositions associated with leaves showed no or weak correlations with the similarity in abiotic and biotic characteristics of the plots, both considering all samples of bacteria and fungi associated with plants (Fig. 3A and C, Supplementary Information 2) or those associated with one of the focus species (Fig. 3B and D, Supplementary Information 2). As an exception, communities of leaf associated fungi showed moderate responses to the biotic environment of the plots (Fig. 3D). In



**Figure 2.** Correlations between the community similarity and the spatial distance between the communities considering bacteria (blue bars) and fungi (orange bars) associated with leaves of all species (A, marked with L below the bars) and with leaves of one of the three focus plant species (B, marked with Cs for *Campanula scheuchzeri*, Od for *Oxyria digyna* or Tb for *Trifolium badium* below the bars), or soil bacteria and fungi (A, marked with S below the bars), or plant species (A, green bar). Bars denote observed Mantel  $r$ -values; the circles denote Mantel  $r$ -values from null model expectations with 95% confidence intervals. The numbers below bars denote effect size Cohen's  $d$ .



**Figure 3.** Correlations between the community similarity and the similarity of environmental parameters based on abiotic properties (A, B) or plant species composition (C, D) considering bacteria (blue bars) and fungi (orange bars) associated with leaves of all species (panels A and C, marked with L below the bars) and with leaves of one of the three focus plant species (panels B and D, marked with Cs for *Campanula scheuchzeri*, Od for *Oxyria digyna* or Tb for *Trifolium badium* below the bars), or soil bacteria and fungi (panels A and C, marked with S below the bars), or plant species (A, green bar). Bars denote observed Mantel  $r$ -values, the circles denote Mantel  $r$ -values from null model expectations with 95% confidence intervals. The numbers below bars denote effect size Cohen's  $d$ .

contrast, communities of soil microbes and plants found in plots with similar abiotic and biotic characteristics were clearly more similar in their composition compared with those communities found in different environments (Fig. 3A and C, Supplementary Information 2). Abiotic environmental factors were more similar between plots in close proximity compared with plots in larger distances (Mantel statistic based on Pearson's product-moment correlation:  $r = 0.32$ ,  $P = 0.001$ ).

### H3: environmental filter

Plant species identity turned out to be a strong predictor for bacterial and fungal communities associated with leaves (Fig. 4, Supplementary Information 2), an effect that was even more pronounced when the distance-based redundancy analyses were restricted to the three focus plant species (Fig. 4B and C).

### H4: interaction filter

Observed mean co-occurrence of OTU/species pairs was lower than null model expectations in all communities tested, i.e. observed C-scores were higher than C-scores obtained from null models (Fig. 5A, Supplementary Information 2). Overall, segregation of bacterial and fungal OTU pairs was higher in soil samples than in most leaf samples. Plants showed strongest segregation.

### H5: subset hypothesis

The proportion of leaf-associated OTUs that are found in both leaf and soil samples was higher when leaf and soil samples originated from the same plot than the mean proportion of shared OTUs when leaf and soil samples did not originate from the same plot (null model expectation, Fig. 5, Supplementary Information 2). However, observed proportion of overlapping bacterial and fungal OTUs was within the 95% confidence interval from null model expectation.

## DISCUSSION

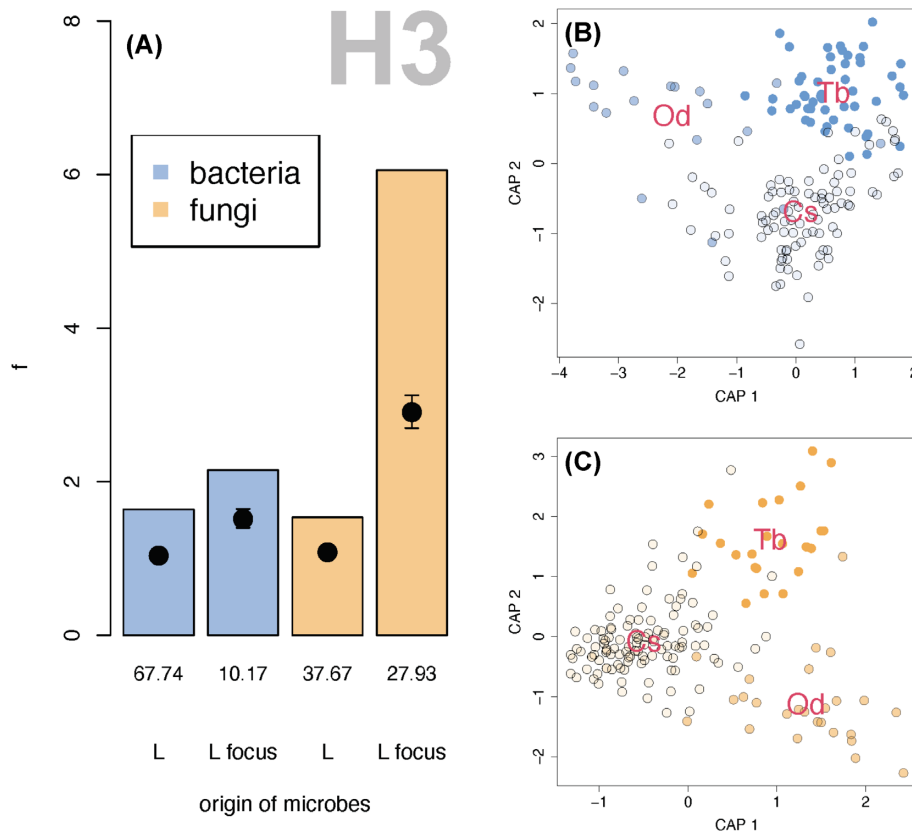
The assembly of local communities is shaped by the interplay of different mechanisms that determine the occurrence, co-occurrence and diversity of species. In our approach, we operationalized the individual processes that contribute to community assembly by deducing specific predictions and testing them on data sets on five community types sampled in the same landscape: bacterial and fungal communities colonizing soil or associated with leaves, and plant communities. Our results show that plant communities contain the strongest spatial signal, followed by microbes colonizing the soil; similarity of plant-associated bacterial and fungal communities was independent of spatial distance. Likewise, plant and soil microbe community compositions strongly responded to the environment whereas plant-associated microbes did not or only weakly. However, we found plant species-specific microbial communities associated with the leaves, supporting the notion that leaf characteristics constitute the environmental conditions for these microbes (Junker and Tholl 2013). The observed co-occurrence patterns deviated slightly positively from null model expectations in all community types indicating that these patterns are either the result of random species distributions or that antagonistic interactions led to the segregation of species. Finally, we identified some bacterial and fungal OTUs that occurred in both soil and leaf samples, but the proportion of leaf-associated OTUs that were also detected in soil samples was low and was not higher

in cases when the leaf and soil sample originated from the same plot. These results suggest that soil microbial communities and plant communities are shaped by dispersal limitation and/or environmental filtering. In contrast, leaf-associated microbial communities are not dispersal limited and are largely buffered from environmental conditions; instead leaf characteristics replace environmental parameters and strongly affect the community composition of bacteria and fungi associated with leaves. The interaction filter seemed to be relaxed for all community types in our study area.

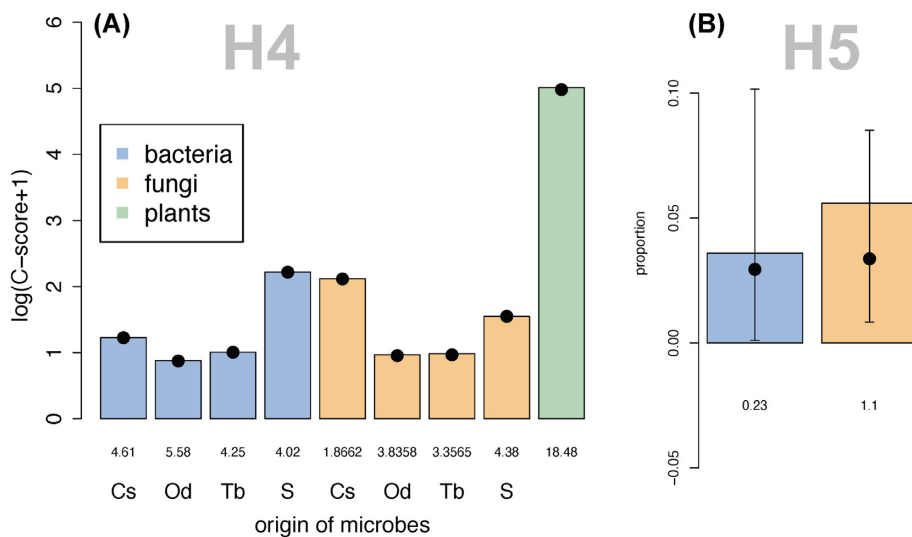
In principle, microbes are less dispersal limited than 'macrobes', which explains why environmental filtering often is the dominant process in microbial community assembly (Van der Gucht et al. 2007; Martiny et al. 2011; Lindstrom and Langenheder 2012; Zhang, Bell and Zhang 2019). Our results on dispersal limitation of the five community types reflect the propagule size of the organisms: seeds are larger than fungal spores that are larger than bacterial cells. This suggests that airborne dispersal is mostly shaping the distribution of bacteria, fungi and plants in our study system. Next to the dispersal abilities of the organisms, the exposure of the habitats to the environment can determine whether dispersal is shaping community assembly. Leaves are more exposed to long-distance dispersed microbes than soil that is less exposed to wind and rain, which may explain why soil microbial communities appeared to be more dispersal limited than leaf communities.

Soil microbial communities and plants completely depend on the water and nutrient availability as well as on chemical and physical properties of the soil they are living on, which is reflected by the strong observed environmental filtering in these communities. Many of these soil properties are modified by the plant species using the soil as substrate (Bulgarelli et al. 2013) explaining why soil microbial communities strongly responded to the plant communities on the plots. Leaf-associated microbes live in their own environment characterized by low availability of nutrients and water, plant metabolites and strong radiation (Vorholt 2012). Additionally, microscopic surface wetness on leaves may create additional niches for specific microbes (Orevi and Kashtan 2021). Thus, the leaf surface habitat is buffered from the environmental conditions experienced by soil microbes and plants. Accordingly, not the environmental conditions on the plot but plant species identity, which is a proxy for differences in leaf properties and thus the niches provided by leaves, strongly affected the composition of the leaf microbiome.

All community types appeared to be more segregated than expected by chance, which may indicate that antagonistic interactions such as competition or inhibition are the dominant factor in the species interactions in the communities observed here. Recently, Blanchet, Cazelles and Gravel (2020) discussed that co-occurrence patterns are poor proxies for species interactions. For instance, shared or exclusive niches may lead to aggregation or segregation, respectively, independently of direct interferences between organisms. Particularly in microbial communities where interactions are often restricted to other microbes in direct proximity (Cordero and Datta 2016; Dal Co et al. 2020), co-occurrence patterns may be not indicative for interactions also because one sample integrates over a number of niches. Even though these results may not be conclusive for the type and strength of interactions, the fact that all community types were more segregated than expected by chance suggests that at least strong facilitation is not common in these communities. Finally, soil seems not to be a major source for microbes associated with leaves although some microbial strains were found in both soil and leaf samples. We collected bulk soil and not specifically the



**Figure 4.** Composition of microbial OTUs associated with leaves is explained by plant species identity. Bars denote observed *f*-values of distance-based redundancy analyses using Bray–Curtis distances followed by permutation test under reduced model; the circles denote *f*-values from null model expectations with 95% confidence intervals. The numbers below bars denote effect size Cohen's *d* (A). Either all leaf samples of bacteria (blue bars) and fungi (orange bars) are considered (marked with L below the bars) or only samples of the three focus plant species (marked with L focus below the bars). Ordination based on distance-based redundancy analyses using Bray–Curtis distances of bacterial (B) and fungal (C) communities associated with leaves of the three focus plant species. Centroids of the three communities are indicated by Cs for *Campanula scheuchzeri* (very pale colors, black frame), Od for *Oxyria digyna* (pale colors, gray frame) or Tb for *Trifolium badium* (saturated colors, no frame).



**Figure 5.** Mean co-occurrence between pairs of bacterial OTUs (blue bars) and pairs of fungal OTUs (orange bars) associated with leaves of the three focus plant species (A, marked with Cs for *Campanula scheuchzeri*, Od for *Oxyria digyna* or Tb for *Trifolium badium* below the bars), or found in soil (panel A, marked with S below the bars), or plant species (green bar). Bars denote observed C-scores; the circles denote C-scores from null model expectations with 95% confidence intervals (note that 95% confidence intervals are too small to be visible). C-scores of null models were lower than observed C-scores in all cases. The numbers below bars denote effect size (A). Mean proportional overlap between leaf and soil OTUs (B). Bars denote mean observed proportion of OTUs that are found on the leaf and the soil of the same plot; the circles denote the proportional overlap from null model expectations with 95% confidence intervals. The numbers below bars denote effect size Cohen's *d* (B).



rhizosphere of individual species, which may have caused the low overlap of soil and leaf microbes in our samples.

The approach to dissect community assembly into separated processes or ‘filters’ that act hierarchically on the regional species pool and shape local species assemblage has been criticized (Gilbert and Bennett 2010; Stegen and Hurlbert 2011; Kraft et al. 2015; Stegen et al. 2015; Cadotte and Tucker 2017; Blanchet, Cazelles and Gravel 2020). As detailed by these authors, the outcomes of our predictions deduced from assembly hypotheses may be the result from the process under consideration, or the result from another process that is overlaying the other one. Shared niches among species will lead to a strong signal in the environmental and the interaction filter. Vice versa, strong mutualistic interactions leading to high co-occurrence may be misinterpreted as a result of environmental filtering. Furthermore, in our study site the effects of the environmental and the dispersal filters do not act independently as abiotic conditions and geographic distance covary along a successional gradient. Finally, composition data alone may not carry sufficient information to infer assembly processes. Thus, the observed pattern for soil microbes and plants cannot be attributed specifically to one of these filters, but most likely they jointly contribute to the findings reported here. Therefore, our analysis may not be suitable to identify individual processes that dominate the assembly of one of the communities observed. However, our comparative approach considering different organisms that live on different substrates but within the same landscape and recorded in close proximity in the same plots is well suited to highlight the characteristics of each of the community types and how this affects their assembly. In summary, the differences in community assembly processes can be attributed to the size of the organisms’ propagules and thus their dispersal abilities, the exposure of the habitats to the environment (i.e. the accessibility to airborne propagules) and the characteristics of the habitat itself (i.e. soil versus leaves). Additionally, the spatial scale and thus the heterogeneity of niches within a sample affect most of the processes discussed in this study: Microbial propagules experience less dispersal limitations than plant propagules on the landscape scale. However, a few millimeters in the soil or on leaves may represent a strong barrier for resident microbes that rely on specific niches. Finally, as discussed above, a one-square-meter plot hosts a community of potentially interacting plants within a shared niche; a soil of leaf sample contains multiple niches with distinct microbial communities. Our study identified organismal traits and abiotic factors that may affect community assembly and may thus stimulate further work on assembly processes conditional to the characteristics of the organism, the habitat and the spatial scale under consideration.

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## SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](https://www.femsec.org/) online.

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