

Research Article

# Contribution of neutral processes to the assembly of microbial communities on *Phragmites australis* leaf litter

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## Abstract

*Phragmites australis* is a remarkable aquatic plant known for its adaptability, wide ecological range and extensive presence in natural wetlands. When combined with its microbiome, it holds unique potential to enhance the overall functionality of wetland ecosystems. To fully harness this potential in both natural and constructed wetlands, it becomes crucial to understand the dynamics of decomposition regarding the substantial biomass generated by *P. australis*. However, our understanding of the selective and neutral processes that shape the microbial communities responsible for decomposing *P. australis* litter remains somewhat limited. In this context, our research reveals that the majority of microbial taxa inhabiting *P. australis* leaves and litter follow neutral distribution patterns, indicating they are less likely to be specifically adapted to the host plant or habitat. Their presence in the community primarily results from their prevalence in the broader metacommunity and source pool. Nonetheless, this should not be interpreted as these taxa being functionally unimportant or lacking close interactions with their host. Instead, the host environment does not differentially select them, and as a consequence, their distributions are shaped predominantly by neutral processes of dispersal and drift.

## Keywords

Bacterial communities, fungal communities, decomposition, freshwater ecosystem, ephemeral lake

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## Pomen nevtralnih procesov pri oblikovanju mikrobnih skupnosti na listnem opadu vrste *Phragmites australis*

### Izvleček

*Phragmites australis* je izjemna vodna rastlina, znana po svoji prilagodljivosti, širokem ekološkem razponu in obsežni prisotnosti v naravnih mokriščih. V kombinaciji s svojim mikrobiomom ima edinstven potencial za izboljšanje splošne funkcionalnosti mokriščnih ekosistemov. Za izkoriščanje njenega potenciala v naravnih in zgrajenih mokriščih pa je ključnega pomena razumevanje dinamike razgradnje velike količine biomase, ki jo ustvari *P. australis*. Naše razumevanje selektivnih in nevtralnih procesov, ki oblikujejo mikrobnih združbe, odgovorne za razgradnjo listnega opada *P. australis*, je še vedno nekoliko omejeno. V tem kontekstu naša raziskava razkriva, da večina mikrobnih taksonov, ki naseljujejo liste in listni opad *P. australis*, sledi nevtralnemu vzorcem porazdelitve, kar kaže, da je manj verjetno, da so posebej prilagojeni gostiteljski rastlini ali habitatu. Njihova prisotnost v združbi je predvsem posledica njihove razširjenosti v širši metaskupnosti in izvornem naboru vrst. Kljub temu to ne pomeni, da so ti taksoni funkcionalno nepomembni ali da nimajo tesnih interakcij z gostiteljem. Le njihovo okolje ne vrši močnega selektivnega pritiska nanje, zaradi česar njihovo porazdelitev oblikujejo pretežno nevtralni procesi razširjanja in ekološkega zdrsa.

### Ključne besede

Bakterijske združbe, glivne združbe, razgradnja, sladkovodni ekosistem, presihajoče jezero

## Introduction

*Phragmites australis* is a perennial grass-like aquatic plant known for their remarkable adaptability and wide ecological range. It has the capacity to establish dense, dominant communities within aquatic ecosystems and is among the most prevalent plant species found in wetlands (Kowalski et al., 2015; Stottmeister et al., 2003). Given their extensive presence in natural wetlands and widespread use in constructed wetlands, *Phragmites* and its microbiome possess distinctive potential for enhancing the overall functionality of wetland ecosystems. Nevertheless, in order to harness its potential in natural and constructed wetlands, it is important to understand also the decomposition dynamics of the vast biomass produced by *P. australis*.

The decomposition process within wetlands can be influenced by a range of factors, with its hydrology and temperatures playing pivotal roles (Dolinar et al., 2016; Serna et al., 2013) in the formation of microbial communities decomposing the plant litter. Apart from climatic conditions, the composition of litter and site elements, specifically the nutrient content within plant litter, may also substantially influence decomposition rates (Alfredsson et al., 2016; Sistla et al., 2012). This is because plants in nutrient-deficient environments often produce litter of

lower quality, which consequently slows the decomposition processes (Rejmánková & Houdková, 2006).

Although the list of potential factors influencing microbial communities is extensive, they can be categorized into two primary groups: selective processes, where microbes thrive within an environment due to differences in their ecological fitness, and neutral processes, which encompass passive dispersal dynamics and the impacts of ecological drift (Chase & Myers, 2011). Although much research is focused on interactions between microbes and their environment, the relative contributions of neutral processes in shaping microbial communities associated with hosts have been mainly overlooked. In contrast, these processes have been subjects of study within the broader field of ecology for many decades, experiencing a renewed surge of interest in recent years (Burns et al., 2016; Cao et al., 2019; Heys et al., 2020).

The neutral theory derives its name from its core assumption of species having equivalent per-capita growth, death, and dispersal rates, assuming species are ecologically 'neutral' in their fitness. Without differences in these factors, community assembly is governed by stochastic processes involving dispersal and drift. Within this framework, organisms within a community are randomly lost over time and are replaced by individuals randomly

either from within the same community or through the dispersal of individuals from an outside community.

Despite simple assumptions of ecological equivalence at the base of neutral models, they have proven remarkably successful in predicting the structures of communities, including microbes (Burns et al., 2016; Cao et al., 2019; Heys et al., 2020). These models find utility in modelling microbial systems where the vast diversity of communities makes it challenging to characterize the specific ecological traits of each taxon. Moreover, they enable researchers to quantify the significance of processes that are challenging to observe directly, such as dispersal, despite their potentially substantial impacts on microbial communities (Kerr et al., 2002; Shen et al., 2018).

This study aimed to build on the previous analysis of fungal communities on decomposing *P. australis* leaves and to evaluate the relative impact the ecological factors and neutral model processes have on the microbial communities on these leaves. Our previous study Likar et al. (2022) showed that a complex network of fungi forms already in the senescent leaves of *P. australis* and persists to the decomposition phase. Furthermore, it seems that habitat has a lower impact on the formation of the community during the early decomposition phase than on the interaction between its members.

In the present study, we assess the ability of neutral models to explain the distribution of microorganisms among a population of decomposing *P. australis* leaves and then determine the conditions leading to departures from neutral behavior. If a reduction in the fit of the neutral model reflects heightened selection pressures, we assumed that deviations from the neutral predictions should show distinct compositional changes in the communities, particularly in cases where ecological traits are conserved. By investigating these hypotheses, we aim to establish a framework for identifying communities and taxa that might be of particular interest based on the extent to which they deviate from the expectations set forth by neutral theory.

## Materials and Methods

### Study location and experimental conditions

The lake Cerknica experiences cyclical inundation, submerging its surroundings for approximately nine months each year. Within this dynamic ecosystem, *P. australis*, a

robust plant species, thrives in diverse aquatic environments, encompassing both the inner lake region and the periphery bordering the lake's tributaries (Longhi et al., 2008).

The experimental conditions followed the procedure described in (Grašič et al., 2022). In brief, we gathered both the upper and lower leaves of *P. australis* during the conclusion of the vegetation period when the plants retained their active. We collected leaves from different parts of the plant to cover the possible microbial diversity. Subsequent to collection, the plant material underwent air-drying at room temperature until a consistent weight was achieved. For the decomposition experiment we used litter bags (1 mm × 1 mm plastic mesh) containing 4 g of the plant material. Water level data were monitored from the nearby hydrological station at Gorenje Jezero-Stržen. In order to minimize direct contact with the substrate, the litter bags were affixed to wooden poles. The decomposition phase of our experiment spanned a duration of 45 days, after which the samples were collected and subjected to air-drying until a consistent weight was reached. Following the drying process, we separated the plant material from non-plant material and processed them for metagenomic analysis.

### Metagenomics

In the present dataset, we analysed the sequences obtained by shotgun sequencing (see Likar et al., 2022 for details), deposited at MG-Rast depository under project mgp97071, libraries mgm4915122.3-mgm4915169.3. In short, whole community DNA from common reed leaves was used for shotgun sequencing (Illumina HiSeqX, 2 × 150 nt pair-end, TruSeq Nano kit). The analysis and annotations were executed on the Metagenomics Rapid Annotation (MG-RAST) online server [22], with the default parameters. Taxonomic identification in this analysis utilized an E-value threshold of  $1e^{-5}$ . All sequencing data are openly available on the MG-Rast server.

### Plant litter elemental analysis

Plant litter was analysed using X-ray fluorescence spectrometry (XRF). Five samples of each treatment were used for multielemental analysis as described in (Grašič et al., 2022). In short: From 100–500 mg of dried and powdered plant material was pressed into pellets using a pellet die and a hydraulic press.  $^{55}\text{Fe}$  (25 mCi; Isotope

Products Laboratories, Valencia, PA, USA) was used as the primary excitation source for the analysis. The analysis of the X-ray spectra was carried out using an iterative least-squares programme, as included in the quantitative X-ray analysis system software package (Vekemans et al., 1994). The quality assurance for the element analysis was determined using standard reference materials: NIST SRM 1573a (tomato leaves as a homogenised powder), in the form of pressed pellets.

## Statistics

All analyses were performed in R (v4.3.1).

To estimate the effect of environment and geo-location on the composition of fungal and bacterial communities, were performed variation partitioning using *vegan* v2.6-4 library. Geographical distances were transformed to rectangular data using principal coordinates of neighbourhood matrix (PCNM) before the analysis. Prior to analysis, the environmental factors and PCNM vectors were subjected to forward stepwise redundancy analysis to reduce the number of variables used in the variation partitioning.

Null models are an essential tool for assessing the issue of multiple assembly processes by mimicking the consequences of random processes, therefore we calculated a modified stochasticity index as describe in (Liang et al., 2020) using *NST* v3.1.10 library. The null communities are generated by randomizing the observed community structure 1000 times based on a null model algorithm described previously (Stegen et al., 2013).

Adherence to the Sloan neutral model was calculated using the R code published by (Burns et al., 2016). Accordingly, the OTUs were grouped into three partitions based on whether they occurred more frequently than ('above' partition), less frequently than ('below' partition), or within ('neutral' partition) the 95% confidence interval of the neutral model predictions. For meaningful comparisons among partitions, we rarefied each partition to an equal number of OTUs, matching the size of the smallest partition.

Furthermore, we compared the fit of the neutral model to that of a binomial distribution model to ascertain whether incorporating drift and dispersal limitations improved the model fit beyond random sampling from the source metacommunity (Sloan et al., 2007). The binomial distribution model represents the scenario where local communities are random subsets of the metacommunity in the absence of drift and dispersal limitations. To

compare the fit of the neutral and binomial models, we examined the Akaike information criterion for each model. Computation of the Akaike information criterion was performed in R, and 95% confidence intervals for all fitting statistics were generated using bootstrapping with 1000 bootstrap replicates.

## Results and Discussion

Step-wise RDA of explanatory datasets selected P concentration, PCNM1 and PCNM2 for fungal community dataset, whereas complete models with all environmental parameters and PCNMs were selected for the bacterial community dataset.

Variation partitioning for fungal communities explained 56% of the overall variation (Fig. 1). Measured environmental parameters explained only 1.5% alone and additional 10.7% in combination with geographical location. Geographical location alone explained the largest portion of the variability. Measured environmental parameters were a little better predictor for bacterial communities and explained 16% when not controlling for geographical location. In contrast to fungal communities, geographical location alone did not explain any variation in the bacterial communities, which showed a very high percentage of unexplained variation.

As measured and unmeasured environmental parameters seem to explain only around 15-55% of the total variation in the microbial communities, we examined the importance of neutral processes on the formation of these communities.

Comparing the number of OTUs shared between fresh and decomposing leaves showed that the largest number of OTUs was specific for all three treatments (Fig. 2). This shows the importance of the initial phyllosphere communities on the formation of microbial communities during the decomposition process.

The same was true for the enriched and under-enriched fungal OTUs, suggesting that these represent ubiquitous generalist that start as colonisers of fresh leaves and after leaf-fall proceed to decomposition of leaf litter. The second largest group of OTUs in the present study was characteristic for dryer habitats t.i. fresh leaves and leaves decomposing in the dry habitat. Similarly, identification of indicator species yielded a large portion of fungal taxa that were indicative for both the fresh leaves and leaves

decomposing in the dry habitat (Likar et al., 2022). Only a few fungal OTUs were characteristic for a single treatment: *Corynespora* was enriched, whereas *Marasmius* and *Hanseniaspora* were under-enriched on fresh leaves. In addition, *Lycoperdon* was under-enriched on decomposing leaves independently of habitat. Interestingly, none of the taxa that deviated from the neutral model were observed as indicator species for either fresh leaves or leaves decomposing in wet or dry habitat (Likar et al., 2022).

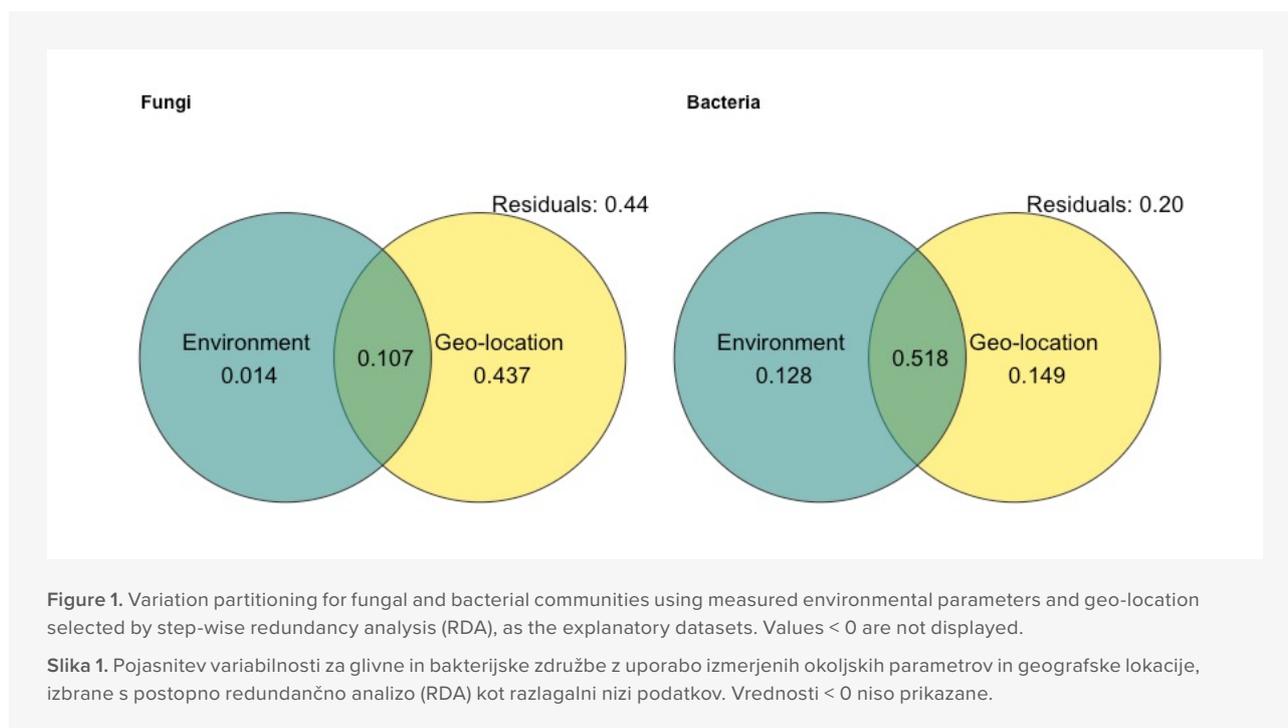
Modified Raup-Crick with Bray-Curtis dissimilarity showed that the local fungal communities on plant litter were more dissimilar than expected by random chance (Fig. 3a). In contrast, local communities on fresh leaves were as dissimilar as expected by the null model. All treatment showed values which indicate that the turnover in fungal communities was governed by drift alone ( $-0.95 < RC < 0.95$ ). To evaluate the stochastic processes in the population, we calculated the modified ST (Fig. 3b). Community assembly was relatively more stochastic on fresh leaves (72% stochasticity ratio (ST)) and in litter decomposing in the dry habitat (68% ST) than in the wet habitat (38% ST). These results suggest that deterministic processes became increasingly important during the decomposition and especially in the wet habitat, where the MST values are under 42%.

In contrast to fungal communities, bacterial communi-

ties in plant litter showed RC values above 0.95, which show a significant departures from the degree of turnover expected when drift acts alone (Chase et al., 2011). Values of  $RC > 0.95$  indicate that dispersal limitation governs observed compositional differences. MST values under 0.5 that were calculated for bacterial communities well support this. Further more, it seems that selection was increased during the transition from fresh leaves to plant litter, as bacterial communities on fresh leaves showed dissimilarity that was well expected by random chance.

While phyllosphere microbial communities of different species exhibit significant dissimilarities (Bao et al., 2019), they all share a similar underlying structure (Wallace et al., 2018). As plants gradually undergo senescence, the variability in phyllosphere microbes tends to incrementally rise (Ferreira et al., 2016), with changes in microbial communities on leaves undergoing decomposition (Kembel et al., 2014; Whipps et al., 2008) increasingly influenced by the leaves' physicochemical properties and competition between the microbes.

Out of on average 244 bacterial families that were observed on plant material in our study, only one family (0.4% of all the families) did not fall into the neutral model and showed enrichment (Suppl. Table S1). None of the bacterial families showed under-enrichment against the neutral model. This would suggest greater importance



of dispersal, as selection would increase or decrease the frequency of bacterial taxa in comparison with neutral model. Nevertheless, unclassified - derived from Rhizobiales group showed frequencies that were above the neutral model. Alphaproteobacteria, which comprised of several symbionts of plants, such as *Rhizobium* as the most abundant group in the roots of *P. australis* He et al.

(2022) and among the top generalists in the phyllospheres of nine perennial plants in a Mediterranean ecosystem (Vokou et al., 2019). Their presence could be beneficial for the plant and provide a nitrogen source for the growth of plants (Sawada et al., 2003) as well as other physiological benefits (Jaiswal et al., 2021), which could explain the departure from the neutral model.

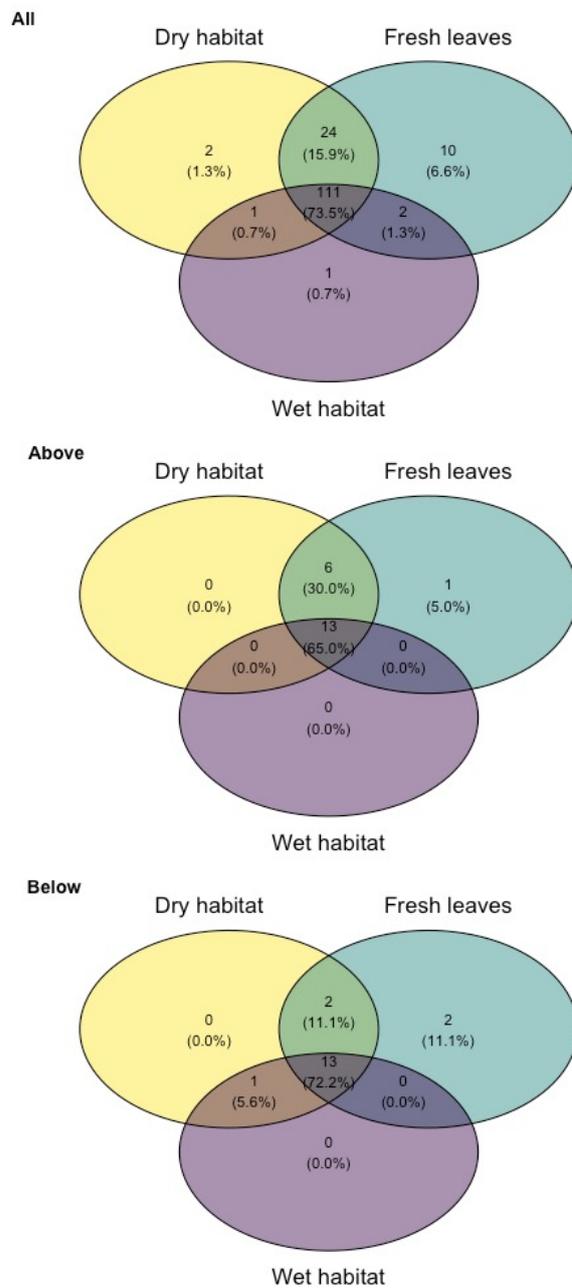
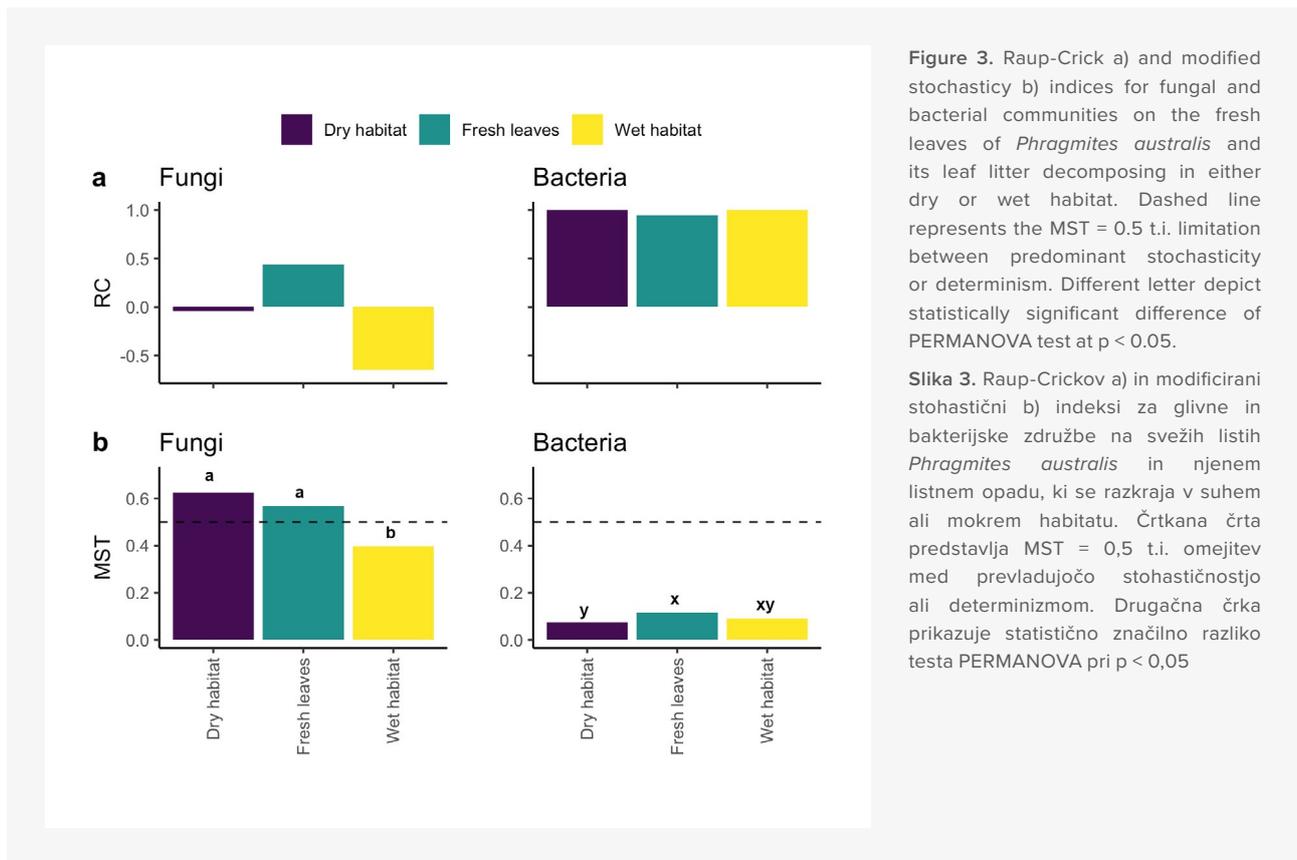


Figure 2. Venn diagrams for fungal communities (All), enriched fractions (Above) and under-enriched fractions (Below) according to Sloan's neutral model for fresh *Phragmites australis* leaves and leaf litter decomposing in either dry or wet habitat. Numbers represent the absolute number of OTUs and percentages represent their proportions regarding all OTUs in the individual representation.

Slika 2. Vennovi diagrami za glivne združbe (All), obogatene frakcije (Above) in podobogatene frakcije (Below) v skladu s Sloanovim nevtralnim modelom za sveže liste *Phragmites australis* in listni opad, ki se razkraja v suhem ali mokrem habitatu. Številke predstavljajo absolutno število OTU-jev, odstotki pa njihove deleže glede na vse OTU-je v posamezni predstavitvi.



In contrast to bacterial communities, fungal communities showed higher taxa numbers that deviated from the neutral model. Overall, the frequency with which fungal taxa occurred in individual communities was well described by the neutral model (Suppl. Table S2, Fig. 4). The migration rate was not very variable and ranged from 0.73-0.89.

The fit of the neutral model was compared with the fit of a binomial distribution, which represents the absence

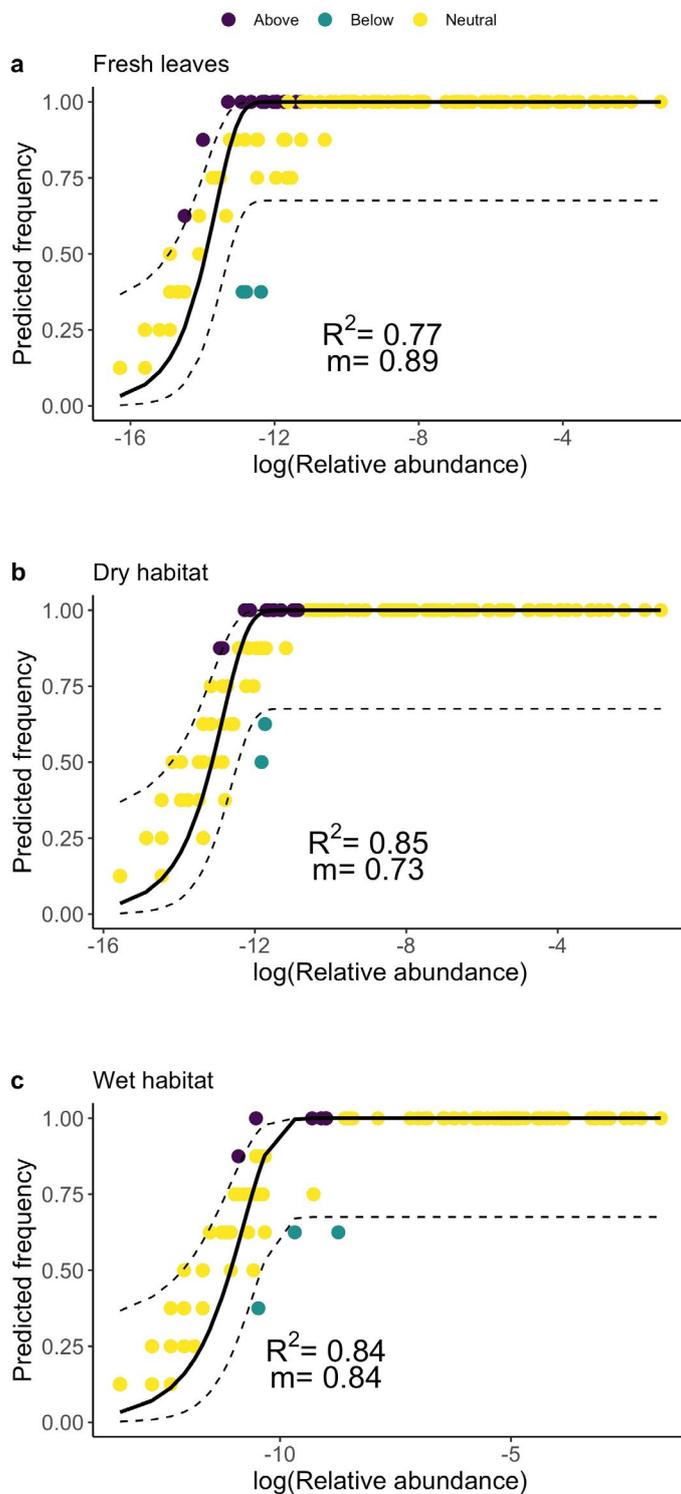
of processes of drift and dispersal limitation using Akaike information criterion and Bayes information criterion (Table 1). We observed a much better fit of the model, if we used the neutral model compared to binomial distribution model. This suggest that the processes of passive dispersal and ecological drift have an impact on the fungal communities. This was observed for fungal communities in various biological systems (Gao et al., 2020; Liu et al., 2023; Zhang et al., 2023).

**Table 1.** Akaike and Bayes information criterions for neutral model and binomial distributions for fungal communities on fresh *Phragmites australis* leaves or leaf litter in either dry or wet habitat.

**Tabela 1.** Informacijski kriterij Akaike in Bayes za nevtralni model in binomske porazdelitve za glivne skupnosti na svežih listih *Phragmites australis* ali listnem odpadku v suhem ali mokrem habitatu.

Plant material/ decomposition habitat	AIC neutral	BIC neutral	AIC binomial
Fresh leaves	-157.059	-151.219	-218.616
Dry habitat	-191.509	-185.655	-223.202
Wet habitat	-219.466	-213.976	7.746

AIC...Akaike information criterion; BIC...Bayes information criterion



**Figure 4.** Fit of the neutral model. The predicted occurrence frequencies for a) fresh leaves and leaves decomposing in either b) dry or c) wet habitat. OTUs that occur more frequently than predicted by the model are shown in purple while those that occur less frequently than predicted are shown in green. Dashed lines represent 95% confidence intervals around the model prediction (solid line). The number represent the model fit (generalized  $R^2$ ) and migration rate ( $m$ ) of the model.

**Slika 4.** Prileganje nevtralnega modela. Predvidene pogostnosti pojavljanja za a) sveže liste in liste, ki se razkrajajo v b) suhem ali c) mokrem habitatu. OTU-ji, ki se pojavljajo pogosteje, kot je napovedal model, so prikazani v vijolični barvi, tisti, ki se pojavljajo manj pogosto, kot je predvideno, pa so prikazani v zeleni barvi. Črtkane črte predstavljajo 95 % intervale zaupanja okoli napovedi modela (polna črta). Število predstavlja prileganje modela (posplošen  $R^2$ ) in stopnjo migracije ( $m$ ) modela.

## Conclusions

Our results suggest that the majority of microbial taxa in the studied ecosystem/plant host system are neutrally distributed and are less likely to be specifically adapted to the host. Therefore their presence in the community is largely the result of their abundance in the surrounding meta-community and source pool. Nevertheless, this does not mean that these taxa are functionally unimportant or even that they are not interacting intimately with their hosts. Rather the host environment is not differentially selecting them, and consequently their distributions are the result of neutral dispersal and drift.

### Supplementary Materials

Suppl. Table S1. Observed frequencies for individual bacterial OTUs and their predicted frequencies under the Sloan neutral model. Upper and lower limits of 95% confidence interval for predicted frequencies are also displayed.

Suppl. Table S2. Observed frequencies for individual fungal OTUs and their predicted frequencies under the Sloan

neutral model. Upper and lower limits of 95% confidence interval for predicted frequencies are also displayed.

### Author Contributions

Conceptualization, M.L. and A.G.; methodology, M.L. and A.G.; formal analysis, M.G. and M.L.; writing—original draft preparation, M.L.; writing—review and editing, M.L., M.G., and A.G.; visualization, M.L.; funding acquisition, A.G. All authors have read and agreed to the published version of the manuscript.

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### Data Availability

All sequencing data are openly available on the MG-Rast depository under project mgp97071, libraries mgm4915122.3-mgm4915169.3.

### Conflicts of Interest

The authors declare no conflict of interest.

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