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Research article

Biotic interactions and environmental modifications determine symbiotic microbial diversity and stability

Zhidong Liu [a,b], Zeguang Guo [a,b], Jin Zhou [a,b], Xuecheng Guo [a,b], Youhua Chen [a,*]

[a] Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Taking amphibians as island models, we examined the effects of interspecific interaction on the diversity and stability of microbial ecological. As skin area increased, the diversity and stability of skin microbes decreased, but the strength of negative interactions increased significantly. In contrast, as gut area increased, the diversity and stability of gut microbes increased, but the strength of interactions remained constant. These results indicate that microbial interactions are affected by habitat properties. When living in fluctuating environments without strong filtering, microorganisms can enhance their negative interactions with other taxa by changing the pH of their surroundings. In contrast, the pH of the gut is relatively stable, and colonized microorganisms cannot alter the gut pH and inhibit other colonizers. This study demonstrates that in the field of microbiology, diversity and stability are predominantly influenced by the intensity of interspecies interactions. The findings in this study deepen our understanding of microbial diversity and stability and provide a mechanistic link between species interactions, biodiversity, and stability in microbial ecosystems.

1. Introduction

Over the past half-century, the debate on diversity and stability has been the focal point of theoretical ecology [1–3], the central interest of which is whether diversity can promote stability. Early theoretical studies suggested that a biologically complex environment was more stable [4–6]. Based on the observation of terrestrial communities, Charles Elton argued that "simple communities were more easily upset than that of richer ones; that is, more subject to destructive oscillations in populations, and more vulnerable to invasions" [5,7].

Through mathematical modelling, well-known theoretical ecologist Robert May questioned these early intuitive ideas [8,9]. May found that ecosystems with high diversity tend to be unstable [9]. This conundrum has remained unsolved for nearly 40 years, despite much evidence suggesting that high-diversity ecosystems in nature tend to be more stable [10]. Ecologists have long been intrigued by how an ecosystem's stability is influenced by its complexity, typically assessed by the diversity of species and their biotic interactions [11–16]. Determining the prevalence of such relationships and discovering the mechanisms behind them is critical to ecosystem management [17].

Theory and experimentation demonstrate that there is a potentially inherent connection between the sum of available resources, interactions between organisms, biodiversity and ecological stability [18,19]. For exmample, there is a well-known pattern for species richness increasing with sampling area as a species-area relationship (SAR) [20–22], which has been observed in many biodiversity studies and experimental research. Through spatial model simulations and bird biomass data validation, Wang et al. found that ecosystem stability also increases with sampling area (IAR), and IAR is largely influenced by species interactions between different sampling areas [23]. Such results again illustrated the influence of available resources and interaction intensity on ecosystem stability. Many hypotheses (e.g., the insurance hypothesis) and experiments suggest that biodiversity can increase ecosystem stability [24]. However, this fundamental question is still debated in microbial research [25–28].

Similar to animals and plants, microbes can influence each other through interactions [29–34]. By studying human-associated microbial communities from different body sites and sponge-associated microbial communities, Yonatan found that highly diverse microbial communities remained stable only when interactions between organisms were weak. [35]. By regulating the intensity of species interactions through nutrient concentrations, Ratzke found that community diversity and stability are

* Corresponding author.
E-mail address: chenyh@cib.ac.cn (Y. Chen).

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related to the strength of species interactions [19]. However, it is worth noting that microbes can change the pH of their surroundings and thus affect other members of the community but animals and plants cannot [36]. This unique indirect interaction excluded more species from the community, leading to loss of biodiversity and destabilization of the microbial community. Therefore, we believe that neglecting the effects of environmental modification by microorganisms on community stability and diversity is one of the potential factors leading to disagreement.

To disentangle the diversity-stability relationship, we need a framework that can not only manipulate the available resources but also eliminate the effect of environmental pH, which can be modified by microbes. Island ecosystems are known as "natural laboratories" for biogeography and evolutionary biology research because of their integrity and simplicity. Larger islands can carry more natural resources and therefore have more habitat and niches to support a greater variety of species [37–39]. Islands are therefore ideal sites for studying the effects of available resources on ecosystem diversity and stability. We can find 'islands' in many forms in macro-ecological researches, such as forested parks in cities and individual thistle plants (which can be seen as a tree island for the arthropods that visit them) in abandoned fields [40,41]. Amphibians are ideal island models due to their semi-isolation, dynamics, finite size, and ability to interact with the surrounding abiotic environment [42]. The amphibian intestine is connected to the stomach and has a relatively stable pH that is not modified by microorganisms. It provides us with a natural experimental site to examine the effects of environmental modification by microorganisms on community stability and diversity.

To understand whether and how available resources and environmental modification by microorganisms affect the complexity and stability of microbial ecosystem. Corresponding to SAR and IAR for plant and animal studies, we constructed microbial diversity-area relationships and stability-area relationships using the skin and gut of amphibians. Our research objectives are the following: (1) By studying the microbial diversity-area relationship (MDAR), we determined whether and how microbial diversity changes with available resources. (2) By studying the microbial stability-area relationship (MSAR), we determined whether and how microbial stability changes with available resources. (3) We assessed the effects of environmental modification by microorganisms on community diversity and stability.

2. Materials and methods

2.1. Sample collection

Different amphibians have different symbiotic microbial compositions. If we use multiple amphibians to construct the network, we may mistakenly filter out some rare microbes due to their low occurrence. Therefore, we chose *Bufo gargarizans* as the study object. Sampling was carried out in May–August 2022. We set up 11 sampling points along the Yellow River's main channel and 10 sampling points along its tributaries. Sampling sites span six provinces, including Shandong (SD), Shanxi (SX), Henan (HN), Gansu (GS), Sichuan (SC), and Shaanxi. We collected *B. gargarizans* of various body sizes to ensure a large body size span. A total of 202 samples were collected. When collecting amphibian samples, we wore sterile gloves to prevent contamination. When collecting skin microbes, we thoroughly rubbed the head, back, side, and abdomen of each animal with multiple sterile swabs that had no bactericidal effect on the bacteria to ensure adequate capture of the complete diversity of microbes present on the given sample (Fig. S1). When collecting gut microbes, we euthanized and dissected the amphibians, and the gut and its contents were collected and stored in 2 ml sterile centrifuge tubes. All gut and skin samples were stored at − 80 °C immediately before the DNA extraction. Our experiments were approved by the Institution of Animal Care and the Ethics Committee of Chengdu Institute of Biology, Chinese Academy of Science (permit no. CIBDWLL2022008).

2.2. Microbial analyses

Following the instructions provided by the manufacturer, we used the MN NucleoSpin 96 Soil kit (MACHEREY-NAGEL) to extract DNA from each sample. After extraction, PCR was started immediately. Using two universal bacterial primers 338 F (5′- ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′- GGACTACHVGGGTWTCTAAT-3′), we amplified the V3-V4 hypervariable regions of the 16 S rRNA gene. Finally, 250 bp paired-end reads were used to sequence the amplicon libraries on an Illumina Nova platform (Nova6000 pe250; Biomarker Technologies). All raw reads in this study were processed using the QIIME2 (v2021.2) software package [43]. Through the DADA2 workflow, amplicon sequence variations (ASVs) were obtained. Following that, all samples were rarefied to the same sequencing depth (28628 reads per sample). We further filtered the feature tables with QIMME2 feature-table filter-features to mitigate the effects of sequencing mistakes and uncommon taxa (p-min-frequency 2 –p-min-samples 2) [44]. The Silva v138 database and the Naive Bayes classifier were applied for ASV taxonomic assignment [45]. Afterwards, we filtered the sequences that were identified as chloroplast and mitochondrial, and the remaining 8861 ASVs were used for downstream analysis.

2.3. Habitat area measurement

To study the microbial diversity-area relationship and stability-area relationship, we need to measure the area of the skin and gut of the sample. There are no uniform standards for measuring skin and gut area in amphibians. In this study, we measured the area using a three-dimensional mathematical model to simulate the sample [42,46]. To evaluate the amphibian skin area, we collected the morphological data of the samples, including head length a, head width b, and body length h. Using these trait values, we were able to construct three-dimensional geometric models (Fig. 1). Specifically, we used the head width as the base diameter and the body length as the height to construct a cylinder to mimic frogs' body part. Then, we used the same head width as the basal diameter and the head length as the height to construct a cone to mimic frogs' head (Fig. 1A). As mentioned above, we calculated the three-dimensional skin surface area (*SKIN*) as follows:

$$SKIN = \frac{\pi b\sqrt{a^2 + \frac{b^2}{4}}}{2} + \pi bh + \frac{\pi b^2}{4} \quad (1)$$

The amphibian gut can be divided into different parts [47]. Through dissection, we found that the volume of the different parts of the amphibian gut varied greatly. To evaluate the amphibian gut area, we constructed a three-dimensional geometric model by dividing the amphibian gut into three parts according to their volume size (Fig. 1B). Specifically, we measured the diameters and lengths of different body

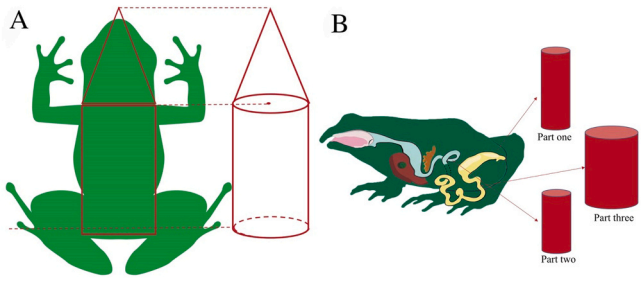

Fig. 1. Geometric transformation and calculation of the microhabitat area size of amphibian hosts. (A) A geometric model for skin microhabitat area surface size calculation; (B) a geometric model for gut habitat area surface size calculation.

parts separately. a_i is the diameter of part i of the gut and b_i is the length of part i of the gut. Then, we constructed three cylinders and obtained the three-dimensional gut area (*GUT*) as follows:

$$GUT = \pi a_1 \times b_1 + \pi a_2 \times b_2 + \pi a_3 \times b_3 \quad (2)$$

2.4. Microbial diversity measurement

To understand how microbial diversity varies with habitat area and construct the microbial diversity-area relationship (MDAR), we evaluated the microbial diversity in the skin and gut of samples. The amplicon sequence variations (ASVs) table we obtained previously contains both species richness (the number of species) and species abundance information simultaneously. In this case, the Hill number becomes the best indicator of microbial diversity measurement. The Hill numbers, initially introduced into ecology by Jost [48] and Chao [49,50], have certain critical advantages over traditional diversity indices. Specifically, according to Hill numbers, diversity was defined as the reciprocal mean of proportional abundance, with taxa weighing themselves differently according to their relative abundances as follows [42]:

$$^qD = \left(\sum_{i=1}^{S} p_i^q\right)^{\frac{1}{1-q}} \quad (3)$$

where ^qD is the diversity in Hill numbers, S is the total number of species in the community, p_i is the relative abundance of species i, and q is the order of diversity. The measure ^qD corresponds to a series of diversity profiles[49,50]. When $q = 0$, ^0D is the number of species in the community. When $q = 1$, ^1D is equivalent to the exponential of Shannon entropy. When $q = 2$, ^2D is equivalent to the inverse of the Simpson index. When q approaches to $\pm\infty$, $^\infty D$ is equivalent to the reciprocal of dominance/rarity indices. The detailed mathematical forms of the three orders of the Hill number are given as follows:

$$^qD = \left(\sum_{i=1}^{S} p_i^q\right)^{\frac{1}{1-q}} = \begin{cases} S, & q = 0 \\ \exp\left\{-\sum_{i=1}^{S} p_i \log(p_i)\right\}, & \lim_{q\to 1} \\ 1/\sum_{i=1}^{S} p_i^2, & q = 2 \end{cases} \quad (4)$$

Following the sequencing of all PCR products, Hill numbers of order 0 to 2 were calculated based on the ASV table to evaluate the diversity of skin and gut microbes.

2.5. Microbial stability measurement

Ecological networks have been widely recognized as important for the anticipation and conservation of ecosystem function and stability [51,52]. Network analysis provides a convincing method to unravel the complex structure of diverse microbial communities over time or space [53]. To investigate the dynamics of MSAR, We categorized the samples into nine groups based on the size of microhabitats (i.e., skin area size) from the smallest to largest (g1–g9, with average skin area ranging from 29.47 cm^2 to 165.05 cm^2 and average gut area ranging from 6.97cm^2 to 92.38cm^2) and then constructed empirical microbial networks (Supplementary Tables 1 and 2). Each empirical microbial network (EMN) contained 20 to 22 samples, and only the ASVs that were detected in at least 10 % of all samples were used for network construction. A standardised criterion ($r > 0.8$, $p < 0.05$) was used to identify significant connections between microorganisms in various networks to ensure network comparability. This is a routine method that has been widely utilized in the literature [54].

To evaluate the overall difference of EMNs, various network topological properties, including the total number of nodes, total number of edges, connectedness, average degree, average path length, diameter, average clustering coefficient, centralization of degree, centralization of closeness, and centralization of betweenness, were computed. By constraining the number of nodes and edges, we randomly connect network nodes and construct 100 random networks for each empirical network to test its significance (Supplementary Tables 3 and 4). The network topology properties are computed for each randomization. The mean and standard deviation of these properties from the 100 randomizations are calculated and compared to the corresponding EMNs. The networks were visualized, with different colours represent different modules. Linear regressions were used to examine the changes in network topological properties as microhabitat areas increased. EMNs were constructed and visualized using the 'ggClusterNet' package [55], and all calculations were performed in R package software version 4.3.0. The overall network stability should depend on the balance of various countering forces owing to differences in network topology [56]. To evaluate whether and how habitat area affects the structure and stability of EMNs, the following topological parameters were also measured.

2.5.1. Modularity

Modularity measures the extent to which the network can be divided into modules. Nodes within a module tend to connect to nodes within the module, with few connections to nodes within other modules. By dampening the impacts of disturbances, complex networks with higher modularity are thought to be more stable [5,57–60]. In this study, modularity was calculated as the ratio of the difference between the modularity of an empirical network and the mean of modularity from the random networks over the mean of modularity from the random networks as follows:

$$Modularity = \frac{M - \overline{M_r}}{\overline{M_r}} \quad (5)$$

where M is the modularity of the constructed network, and $\overline{M_r}$ is the mean of modularity from the random networks.

2.5.2. Number of keystones

Indeed, nodes of high abundance are intuitively important for microbial networks. However, there are also nodes that are less abundant but play an important role in network construction and functioning, and these nodes are referred to as keystones. It has been shown that networks with more keystone taxa are usually more stable, and their removal leads to drastic changes in the network structure and functionality [61–63]. In this study, three kind of keystone taxa including module hubs, connectors and network hubs were identified by calculating the within-module connectivity (Z_i) and among-module connectivity (P_i) value[64,65].

2.5.3. Robustness

Network robustness is a currently recognized measure of microbiotic community stability, which measures the extent to which microbial extinction affects the community by calculating the proportion of species remaining in the network after the removal of nodes. More remaining nodes indicate that the microbial community is less affected by the taxon extinction and more stable. [26,66]. The effects of species removal on the remaining species can be measured as follows:

$$E_i = \frac{\sum_{j\neq i} a_j s_{ij}}{\sum_{j\neq i} a_j} \quad (6)$$

where a_j is the relative abundance of species j and s_{ij} is the association strength between species i and j, which is measured by the Pearson correlation coefficient. After node removal, if there is no node connected to node i ($a_j = 0$) or there are not enough mutually beneficial symbiotic effects to support node i ($s_{ij} < 0$), node i is considered to be removed

from the network. In the present research, network robustness was measured when half of the nodes were removed.

2.5.4. Vulnerability

The vulnerability of each node reflects its contribution to the network's global efficiency, and the vulnerability of a network is indicated by the maximal node vulnerability in the network [67]. Network efficiency describes how quickly information is spread within it, which can be measured as follows:

$$E = \frac{1}{n(n-1)} \sum_{i \neq j} \frac{1}{d(i,j)} \tag{7}$$

where n is the number of nodes and $d(i,j)$ is the length of the shortest path connecting nodes i and j is the number of edges in the shortest path between nodes and. Generally, a less vulnerable network usually suggests a more stable microbial community.

2.5.5. Resistance

Network resistance is defined as the ability of the network to maintain connectivity after the removal of nodes or edges. Similar to network robustness [68], network connectivity is also measured by removing network nodes and then calculating the change in network attributes, which can be derived mathematically as follows:

$$\widetilde{\lambda} = \ln\left(\frac{1}{n}\sum_{i=1}^{n} e^{\lambda_i}\right) \tag{8}$$

where n is the number of nodes and λ_i is the eigenvalue of the network adjacency matrix after node deletion. This index can be intuitively understood as a kind of network information loss or adjacency matrix information loss.

2.6. Microbial interaction strength measurement

If the MSAR and MDAR of amphibian skin and gut microorganisms differ significantly, it indicates that the microbial community can modify their surroundings and then change the interaction pattern of the microbial community, thereby affecting the diversity and stability of the microbial community. Quantifying microbial interactions has always been a challenge due to their complexity, dynamics and the large number of interactions within communities [69]. To address this issue, a new index called cohesion has been proposed to quantify the degree of connectivity of microbial communities based on the correlation between the relative abundance of different taxa in the microbial relative abundance matrix [70]. The higher the positive cohesion index, the greater the positive interaction within the community. Correspondingly, The higher the negative cohesion index, the greater the negative interaction within the community. To verify whether microbial interactions are affected by habitat area, positive and negative cohesion values are calculated for each sample (j) as follows:

$$Cohesion_j = \sum_{i=1}^{n} a_i \overline{r_i} \tag{9}$$

a_i is the abundance of taxon i in sample j, $\overline{r_i}$ is the connectedness which can be calculated by averaging the significant positive or negative correlations of that microbial taxon with the remaining microbial taxa in the network. In short, first, pairwise correlations are measured between all taxa based on the data matrix of relative abundance of taxa × samples, and then the null model-corrected correlations for each taxon are made by removing the pairwise correlations based on the null model (for details, see Ref.[69]). All positive and negative null model-corrected correlations for each sample are averaged to provide a connectivity matrix with average positive and negative correlations for different samples. Finally, positive and negative cohesions are determined for each sample using the prior procedure. Higher absolute values for both negative and positive cohesion (which span from 0 to 1) reflect the degree of cooperative behaviours or competitive interactions.

3. Results

3.1. Community turnover along the habitat area gradient

The distribution of abundance of different species in microbial communities is often unbalanced: a few species are abundant, whereas the majority are rare [71]. Before constructing the network, filtered out species with low abundance and few occurrences. The shifts in taxonomic composition of the microorganisms with habitat area were investigated. Only the taxa that were found in at least 10 % of all samples in each group were included in the analysis to reduce the effects of rare ASVs in the dataset. Skin microbes and gut microbes showed very different patterns. Among the nineteen most abundant skin microbial taxa, fourteen were small skin colonizers (significantly decreased with increasing skin area, namely *Proteobacteria, Bacteroidota, Actinobacteriota, Acidobacteriota, Cyanobacteria, Patescibacteria, Chloroflexi, Gemmatimonadota, Verrucomicrobiota, Desulfobacterota, Deinococcota, Myxococcota, Bdellovibrionota,* and *Methylomirabilota*), and five showed no significant trend (Fig. 2A). Among the twenty-five most abundant gut microbial taxa, seven were big gut colonizers (significantly increased with increasing gut area, namely *Acidobacteriota, Firmicutes, Fusobacteriota, Desulfobacterota, Methylomirabilota, Fibrobacterota,* and *Dependentiae*), and eighteen showed no significant trend (Fig. 2B).

3.2. Microbial diversity-area relationship

Amphibian skin and gut microbial diversity measured using the Hill number was calculated for three orders (q was equal to 0, 1 and 2). By using Eqs. (1) and (2), we measured the amphibian skin and gut area, and then we fit a linear model between microbial diversity and habitat area. The results are shown in Fig. 3, where we can see that the amphibian skin microbial diversity decreased significantly with increasing skin area size with for Hill number with order q= 0 and decreased marginally significantly with skin area with q= 1 (Fig. 3A). In contrast, the amphibian gut microbial diversity increased significantly with gut area with q= 0,1 (Fig. 3B). This is because the amphibian gut is connected to the stomach, the environment is stable in terms of pH and microbes cannot change the pH of their surroundings, while an increase in gut area means more available resources and broader niches, allowing a greater variety of microbes to survive; therefore, gut microbial diversity increases with gut area. In contrast, amphibian skin is fragile and vulnerable to external disturbances, and the larger the skin area is, the more available resources there are, while the microorganisms that initially colonize the skin have sufficient resources to modify their microenvironment, inhibiting the colonization of other microorganisms, a phenomenon that might be related to the priority effect in community ecology [72,73]. Such a result indicated that microorganisms can change the pH of their own surroundings and thus affect the compositional structure of the symbiotic microbial community.

3.3. Microbial stability-area relationship

We constructed nine EMNs separately to unravel the dynamic changes in skin and gut microbial associations with habitat area (Fig. S4 and Fig. S5). The results showed that the EMNs underwent profound changes along the habitat area gradient (Fig. S4 and Fig. S5). All microbial networks are scale-free and non-randomized with the degrees of the nodes exhibited a power-law distribution (Fig. S2 and S3). Along the habitat area gradient, the skin microbial network size (total number of nodes) decreased significantly (R^2=0.75, $P < 0.05$), so did network connectivity (total number of links, L; R^2=0.67, $P = 0.01$), network

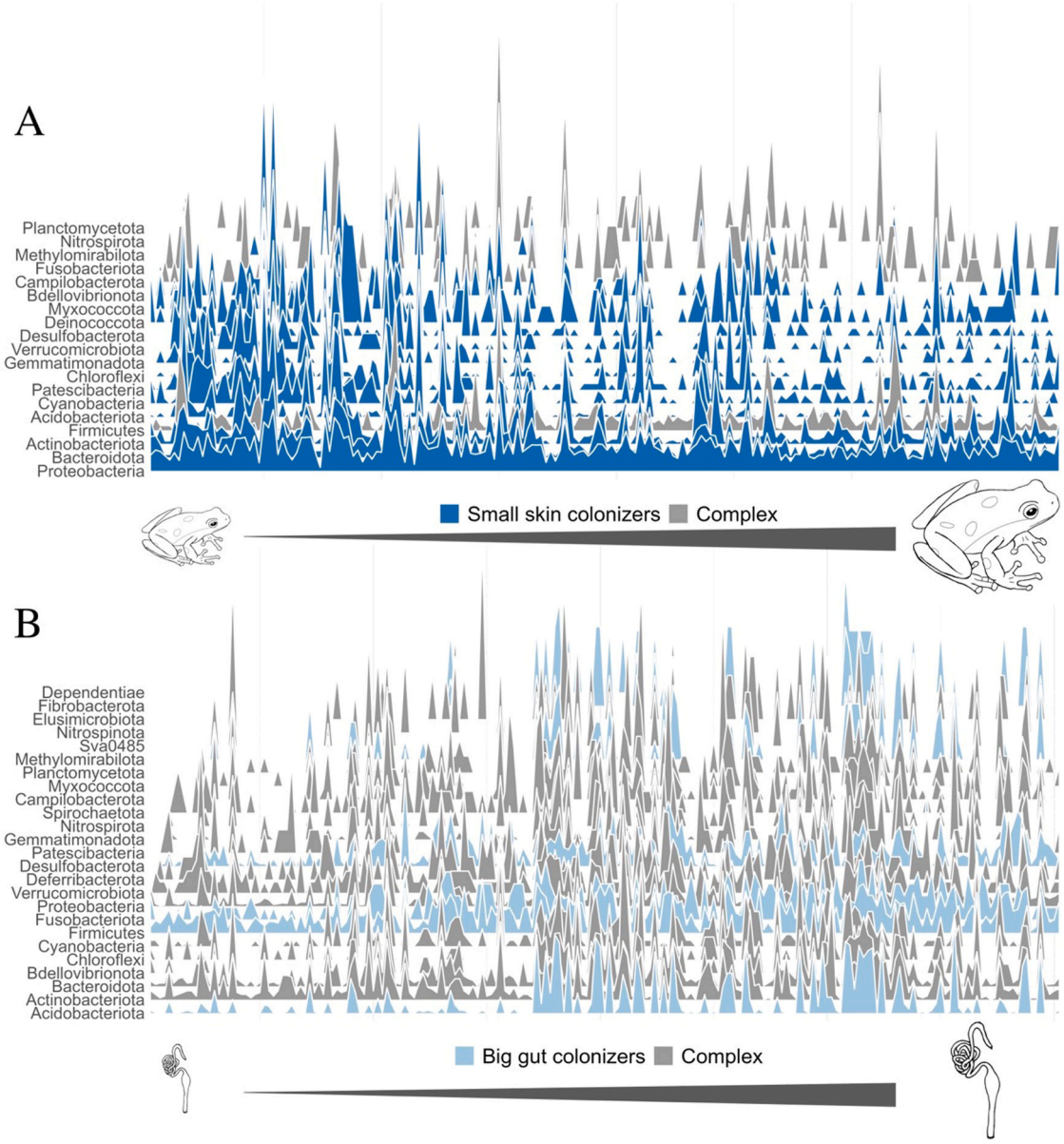

Fig. 2. Dynamics of the relative abundance of the bacteriome with habitat area. Only the taxa that were detected in at least 10 % of all samples per group are shown at the phylum level. (A) The shifts in taxonomic composition of the skin microbial community with habitat area. (B) The shifts in taxonomic composition of the gut microbial community with habitat area. (Small skin colonizers: significantly decreased with increasing skin area; Big gut colonizers: significantly increased with increasing gut area; complex: not changed with habitat area).

diameter (longest distance between any two nodes in the network; R^2=0.87, $P < 0.01$), number of keystone (R^2=0.52, $P = 0.028$) and network modularity (R^2=0.44, $P = 0.059$). In contrast, the gut microbial network size increased marginally significantly (R^2=0.4, $P = 0.07$), as did the network connectivity (R^2=0.67, $P < 0.05$) and the network diameter (R^2=0.36, $P = 0.097$). No significant trends were observed between the network modularity and the number of key nodes with habitat area in amphibian gut microbes (Fig. 4).

In order to assess the impact of habitat area on the stability of microbial networks, a regression analysis was conducted to examine the relationship between changes in network topological parameters and habitat area. Specifically, we simulated species extinction and calculated the robustness (the resistance to node loss) of the EMNs. Familiar patterns reappeared. On the basis of random species loss of module hubs, amphibian skin EMN robustness.random decreased with sample size (R^2 = 0.46, P = 0.046). On the basis of targeted removal of module hubs, amphibian skin EMN robustness.target decreased marginally with sample size (R^2 = 0.43, P = 0.057), and network resistance (the ability to maintain connectivity after node loss) (see Methods for details) decreased significantly in skin EMNs (R^2 = 0.66, P = 0.0082) (Fig. 5A). The robustness.random and robustness.target of the corresponding amphibian gut EMNs increased marginally with sample size (R^2 = 0.43, P = 0.054;R^2 = 0.44, P = 0.052), and the network resistance did not vary with habitat size (Fig. 5B). One unexpected result was that the network vulnerability (the maximum decrease in network efficiency when a single node is deleted from the network) did not vary with habitat area.

3.4. Characterization of interaction strength across the habitat area gradient

To determine whether the sum of available resources and the modifying effect of the microbial community on the surrounding

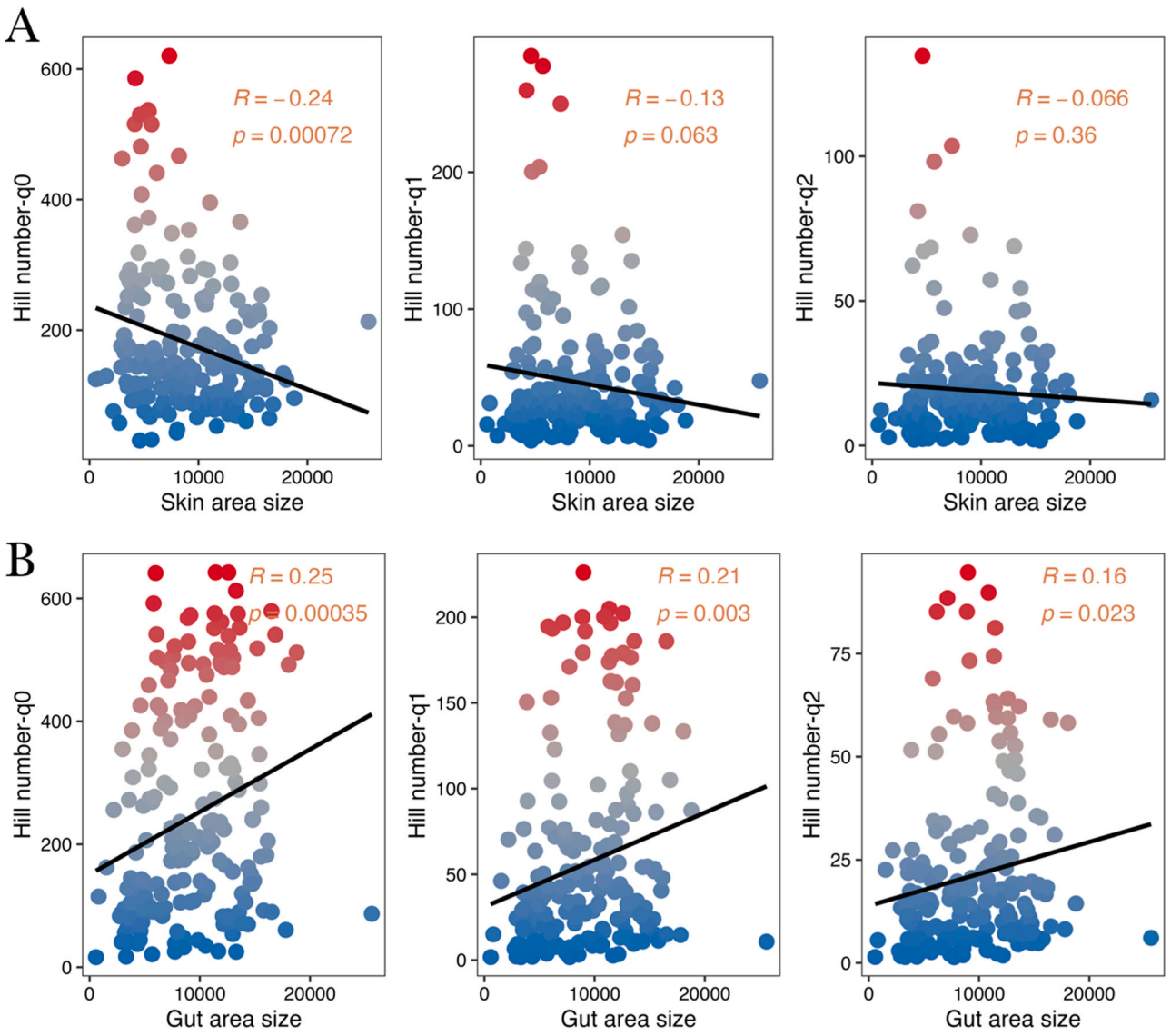

Fig. 3. Overall relationship between symbiotic microbial diversity (measured using the order of Hill number [q = 0, 1, 2, 3]) and habitat area sizes (mm^2). (A) Amphibian skin microbial diversity-area relationship. (B) Amphibian gut microbial diversity-area relationship.

environment change the interaction pattern of the microbial community, we investigated the positive cohesion and negative cohesion of skin and gut microorganisms along the habitat area gradient, respectively. When studying amphibian skin microbes, we found that positive cohesion did not vary with habitat area, while negative cohesion increased with habitat area (Fig. 6A and B). When studying amphibian gut microbes, we found that both positive and negative cohesion did not vary with habitat area (Fig. 6D and E). Combined with the above findings, we can conclude that, in general, as available resources increase and environmental conditions fluctuate, microorganisms enhance their negative interactions with other taxa by changing the pH of their surroundings, thereby reducing the diversity and stability of the microbial community. When living in an environment with nonvariable acid—base properties, microorganisms cannot change the pH of their surroundings, more resources can support more microbial survival, and the microbial community will be more stable.

Furthermore, we calculated the absolute value of negative:positive cohesion, which allowed us to determine whether larger habitat areas are better characterized by processes driving negative interactions, which include competition and niche divergence, than small habitats. We then used linear regression to examine the relationship between habitat area and negative:positive cohesion. We found that the negative:positive cohesion of amphibian skin microbes increased with habitat area, while a similar phenomenon was not observed in gut microbes (Fig. 6C and F). This result suggests that when living in an environment with variable acid—base properties, negative rather than positive strength between microbial taxa dominates as available resources increase. When living in an environment with nonvariable acid—base properties, the pattern of microbial interactions does not change with available resources.

4. Discussion

The relationship between species diversity and stability in ecosystems has been extensively studied in the literature [74]. Robert May demonstrated through mathematical modelling that interactions between organisms play a major role in determining the biodiversity and stability of ecosystems. A major obstacle to confirming this hypothesis is the difficulty of measuring and experimentally manipulating the strength of species interactions. In this study, we introduced a way to manipulate microbial interactions, which allows us to understand how interactions affect the biodiversity and stability of microbial

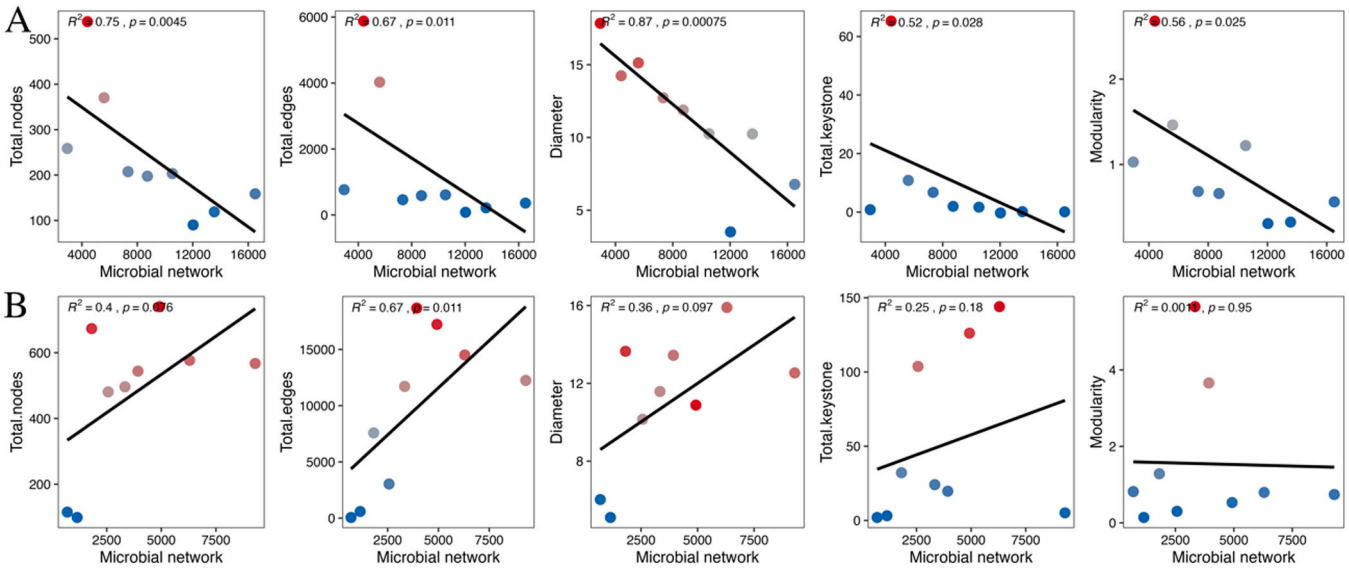

Fig. 4. Correlations between habitat area and network topological properties. (A) Network topological properties of amphibian skin microbes in relation to skin area. (B) Network topological properties of amphibian gut microbes in relation to skin area.

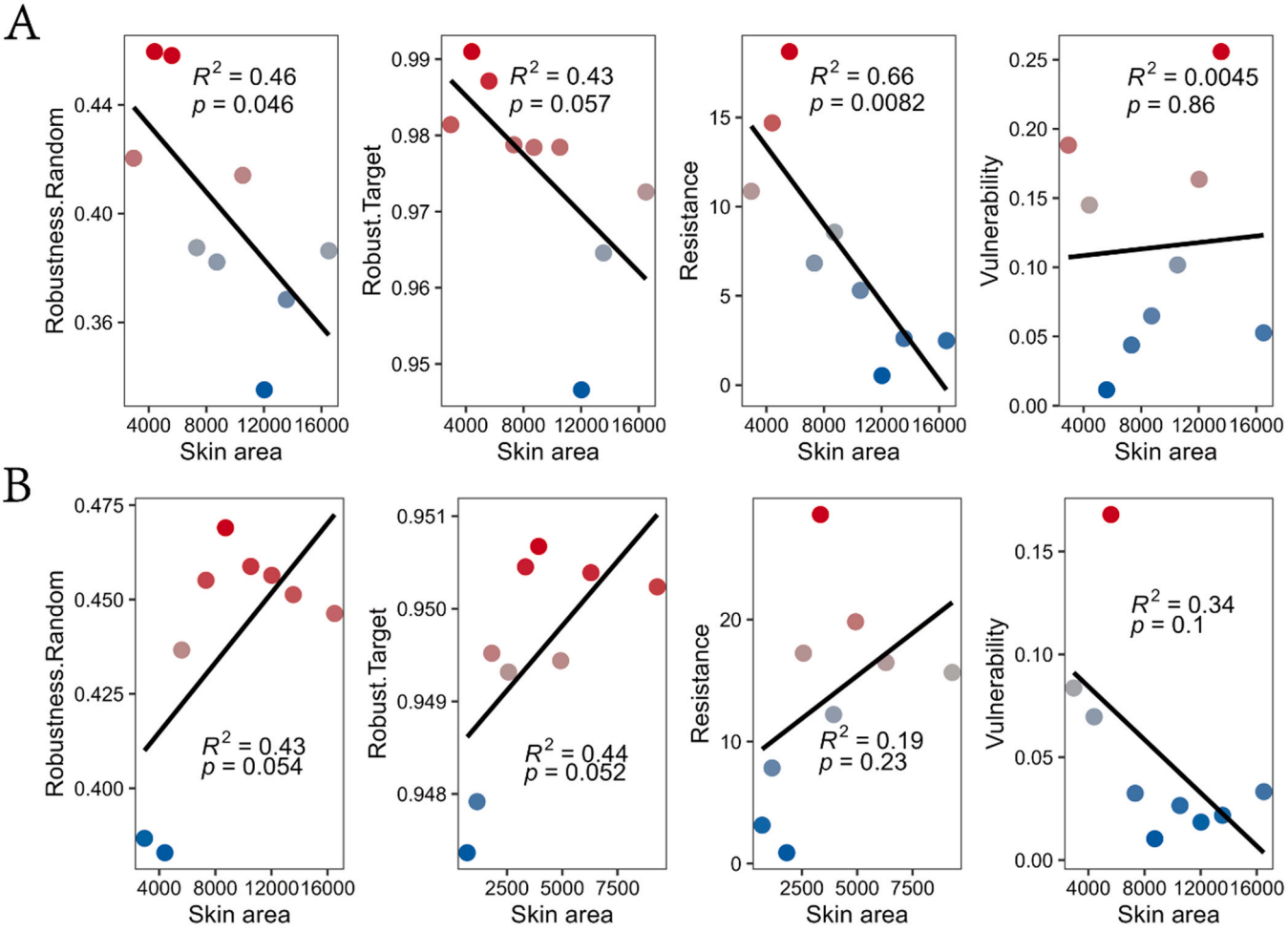

Fig. 5. Trends in network stability along with habitat area. Robustness-random was measured as the proportion of taxa that remained with 50 % of the taxa randomly removed from each network. Robustness-target was measured as the proportion of taxa that remained with certain numbers of key nodes removed from each of the networks. Network resistance was measured as the ability of the network to maintain connectivity after the removal of nodes. Network vulnerability was measured by the maximum node vulnerability in each network.

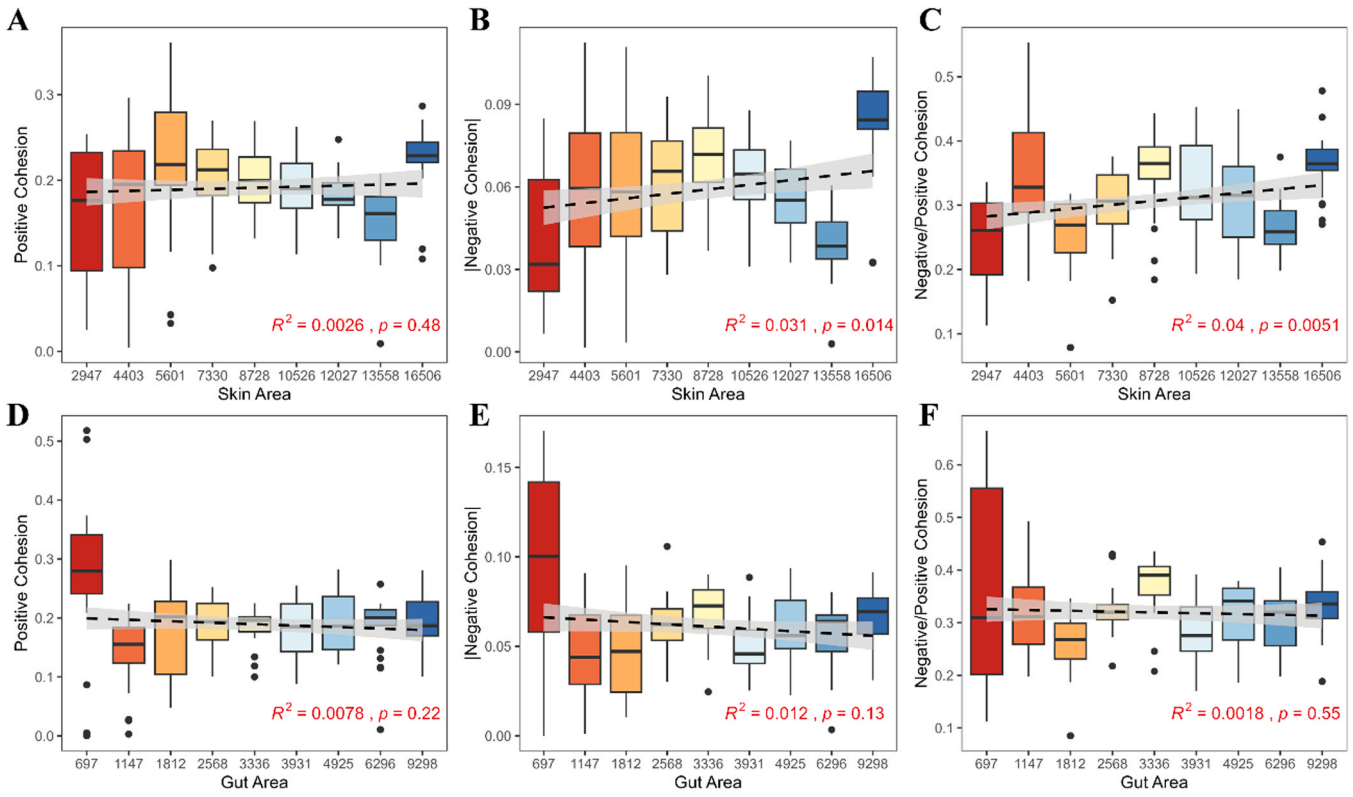

Fig. 6. Cohesion of microbial communities and their relationships with habitat area. (A) Changes in the positive cohesion of the skin microbial community along the skin area gradient. (B) Changes in the negative cohesion of the skin microbial community along the skin area gradient. (C) Changes in the ratio of negative:positive cohesion of gut microbes along the skin area gradient. (D) Changes in the positive cohesion of the gut microbial community along the gut area gradient. (E) Changes in the negative cohesion of the gut microbial community along the gut area gradient. (F) Changes in the ratio of negative:positive cohesion of gut microbes along the gut area gradient.

communities. Interactions between microbes shape ecosystem diversity and stability. When microorganisms colonize an environment with variable acid—base properties (amphibian skin), a larger habitat area causes more negative microbial interactions, resulting in lower community diversity and stability. Correspondingly, when microorganisms colonize an environment with nonvariable acid—base properties (amphibian gut), a larger habitat area has no effect on microbial strength, leading to more diverse and stable communities.

Microbial network vulnerability measures the stability of the information transfer efficiency of microbial networks, which was found to be unaffected regardless of whether the diversity of the microbial community increased or decreased (Fig. 5). We believe that this is because the taxa added or lost in the community are not the backbone of information transfer but are more similar to branches linking the backbone to each other. Notably, the number of keynodes and the modularity of the microbial network decreased with increasing skin area, but were not affected by gut area size (Fig. 4). In summary, we conclude that the structure of microbial networks varies with habitat size, whether the principles of network organisation change is determined by the nature of the microbial habitat.

Notably, microorganisms can prevent other taxa from surviving by changing their own surroundings, whereas plants and animals generally do not have this ability; thus, it remains to be seen how applicable our findings can be outside the microbial world. In a plant community, a similar process for biodiversity loss can be discovered. When growing in a high nutrient environment, plants become taller, resulting in the shading of light and preventing other plants from photosynthesizing, which causes a loss of biodiversity, but the effect on community stability needs further study. In an animal community, interspecific interactions include competition, predation, interactions, symbiosis, and parasitism. Apart from the obvious impact of humans on the environment, which reduces the diversity and stability of biological systems, no other animals have been reported to have similar abilities. Similar to the amphibian gut microbiome, a study discovered that the diversity and stability of North American bird communities grow with area [23].

Laboratory experiments on simple microbial systems have revealed many principles of ecology and evolution. However, it is difficult to determine how far these findings can be transferred to natural, more complex communities because of their simplicity. Our study shows that, using simple pairwise interactions, we can understand the biodiversity and stability of complex systems. There exists a variety of evidence for the connection between habitat area, biodiversity, and stability. A larger habitat area often comes with higher diversity and stability in ecosystems. Our research proves that in the field of microbiology, an increase in interaction strength decreased the microbial community's diversity and stability in terms of building and maintaining community stability. For these ecosystem properties, the mechanistic details of the interactions seem to be as important as available resources.

5. Conclusion

Microbial interactions and their modification of the environment have a significant impact on the diversity and stability of communities. Microbes can inhibit the growth of other taxa by modifying the surrounding environment, thereby reducing the diversity and stability of the community. This provides a possible explanation for the inconsistency of previous research results on the relationship between microbial community diversity and stability and provides a basis for subsequent research on the relationship between the stability and diversity of animal and plant communities.

CRediT authorship contribution statement

Zhidong Liu: Conceptualization, Methodology, Visualization, Writing – original draft. **Zeguang Guo:** Methodology, Writing – review & editing. **Xuecheng Guo:** Data curation. **Jin Zhou:** Conceptualization, Supervision, Writing – review & editing. **Youhua Chen:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2024.05.047.

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