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COMMUNITY ECOLOGY – ORIGINAL RESEARCH

Experimental demonstration of the importance of keystone communities for maintaining metacommunity biodiversity and ecosystem functioning

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Abstract

As local communities within a metacommunity may differ considerably in their contributions to biodiversity and ecosystem functioning, it has been suggested that conservation priority should be given to disproportionately important local communities (i.e., keystone communities). However, we know little about what characterizes a keystone community. Using laboratory protist microcosms as the model system, we examined how the environmental uniqueness and location of a local community affect its contributions to the metacommunities. We found that the removal of local communities with unique environmental conditions, which supported endemic species, reduced regional-scale diversity, qualifying them as regional-scale keystone communities. In addition, the local communities possessing unique environmental conditions had greater impacts on ecosystem functions, including biovolume production and particulate organic matter decomposition. We also found that keystone communities for biovolume production were not keystone for organic matter decomposition, and vice versa. Our study, therefore, demonstrates the important role of keystone communities in maintaining biodiversity and functioning of metacommunities.

Keywords Connectivity · Ecosystem functioning · Heterogeneity · Keystone community · Metacommunity

Introduction

The ever-escalating human activities have resulted in rapid habitat loss and fragmentation worldwide, posing a serious threat to Earth's biodiversity (Vitousek et al. 1997; Dirzo and Raven 2003; Fahrig 2003; Haddad et al. 2015). Habitats in a fragmented landscape, however, are not equal (Myers et al. 2000; Brooks et al. 2006). Some communities may be disproportionally more important than others (e.g., relative to their habitat area or biomass) for the maintenance of community and ecosystem properties of the metacommunity in which they are embedded. Analogous to keystone species, which play a disproportionally important role (relative to

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Xian Yang and Jiaqi Tan have contributed equally to this work.

Our study provides empirical evidence of keystone communities. Using laboratory protist communities as model systems, we show that the loss of keystone communities carries significant consequence for biodiversity and ecosystem functioning at both local and regional scales. Our findings have important implications for the effective allocation of conservation resources.

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their abundance) in regulating local community structure and ecosystem functioning (Paine 1966; Power et al. 1996), these communities could be considered as keystone communities (Economo 2011; Mouquet et al. 2013). The loss of these communities may not only cause the loss of local species diversity but also erode regional biodiversity and ecosystem functions (Mouquet et al. 2013). In contrast, some communities may be considered a burden within metacommunities (Mouquet et al. 2013), and the removal of such burden communities would have a positive effect on community and ecosystem properties of the region. Identifying these keystone/burden communities, therefore, has important implications for the effective allocation of limited conservation resources in increasingly fragmented landscapes (Economo 2011; Mouquet et al. 2013). Nevertheless, empirically we know little about the characteristics of those communities that have large regional impact (Resetarits et al. 2018).

Metacommunity theory suggests that environmental heterogeneity and patch connectivity are important in determining both local and regional biodiversity (Leibold et al. 2004; Holyoak et al. 2005). We, therefore, hypothesize that the characteristics of local patches, especially their contribution to environmental heterogeneity and patch connectivity, may determine whether the communities in these patches are the keystone communities to the metacommunities. The decrease in environmental heterogeneity due to the loss of a patch with unique environmental conditions may have significant impacts on regional biodiversity (Legendre and Caceres 2013). When the dispersal rate among local communities is low or moderate, heterogeneity could enhance regional biodiversity through species sorting, where species differing in their niches utilize different patch types (Leibold 1998; Chase and Leibold 2003), or through source-sink dynamics (Pulliam 1988; Loreau and Mouquet 1999; Mouquet and Loreau 2002), where local populations persist in unfavorable habitats (i.e., sink communities) through migration of individuals from favorable habitats (i.e., source communities) (Mouquet et al. 2006). Under these circumstances, keystone communities are likely to dwell in unique habitat patches that contribute most to environmental heterogeneity and support the positive growth of endemic species populations (Tews et al. 2004; Resetarits et al., 2018). When dispersal rate is sufficiently high, local and regional biodiversity may be depressed as the local communities become homogenized through strong source-sink dynamics (Mouquet and Loreau 2003). In this situation, habitat patches that support regionally dominant competitors or generalist predators may reduce local and regional diversity through competitive exclusion or predation (Mouquet and Loreau 2003; Cadotte and Fukami 2005; Cadotte 2006). A local community that supports the positive growth of competitively dominant species or generalist predators, via unique environmental conditions, may, therefore, be a burden rather than keystone community in metacommunities characterized by strong source-sink dynamics.

The contribution of local communities to the spatial configuration, especially the connectivity, of the metacommunities (Altermatt and Holyoak 2012; Carrara et al. 2014) may also influence their significances to the metacommunities. In particular, the loss of a patch that occupies a central position with connections to many other patches would compromise the overall connectivity of a metacommunity. Reduced connectivity could preclude the operation of source-sink dynamics in a metacommunity and, therefore, reduce the number of local patches colonized by certain species (Pulliam 1988; Loreau and Mouquet 1999; Mouquet and Loreau 2002; Thompson et al. 2017). More specifically, when the presence of species in a sink community is maintained through immigration of individuals from source communities, cutting down the migration would increase the risk of local extinction in the sink community, increasing the risk of stochastic extinction across the whole metacommunity. In this situation, highly connected local communities could be qualified as keystone communities. Alternatively, when strong source-sink effects dominate the spatial dynamics of the metacommunities with extremely high dispersal rates, communities embedded in high connectivity patches might be burden rather than keystone communities. Removing such local communities may increase regional diversity by maintaining local spatial refuges from competitors and predators (Cadotte and Fukami 2005).

Removing local habitat patches may also have consequences for ecosystem functioning. On the one hand, ecosystem functions might be affected when the removal of local patches eliminates the spatial insurance effects of biodiversity, by which ecosystem functions of metacommunities are maintained when species are able to move between patches to track their favorable environments (Loreau et al. 2003a; Gonzalez et al. 2009; Staddon et al. 2010; Shanafelt et al. 2015). Spatial insurance effect requires a combination of species sorting dynamics by which species with different niches are able to disperse to their favorable habitats, and source-sink dynamics by which species could persist in habitats where the environment is unfavorable (Thompson et al. 2017). Both mechanisms could be eliminated when patches that contribute the most towards connectivity and/or heterogeneity are removed. On the other hand, the cross-habitat movements of energy and materials could exert important influences on ecosystem functioning (Polis et al. 1997; Loreau et al. 2003b). In metacommunities where biomass or nutrient moves from productive or fertile patches (i.e., source patches) to unproductive or infertile patches (i.e., sink patches) (Loreau et al. 2003b; Gravel et al. 2010; Mouquet et al. 2013), removing a source patch may reduce ecosystem functions (e.g., primary productivity), while removing a sink patch is likely to enhance ecosystem functions in the neighboring patches or the whole metacommunity (Mouquet et al. 2013). As local patches can be sources or sinks for different materials, and thereby are important for carrying out different functions, focusing on different ecosystem functions might result in different keystone communities. Moreover, loss of keystone communities may impair ecosystem functions indirectly through biodiversity loss, as a large number of studies have reported positive effects of biodiversity on ecosystem functioning at both local (Naeem et al. 2012; Hooper et al. 2005, 2012; Cardinale et al. 2006, 2012) and regional scales (Venail et al. 2010; Grace et al. 2016).

To examine how the environmental uniqueness and location of a local community affect its contributions to the metacommunities, we conducted an experiment using laboratory microcosms consisting of freshwater protozoan communities. In our experiment, each of the metacommunities contained three local communities that included one middle community being lined up with and connected to the other two local communities. We incubated the three local communities under light or dark conditions to manipulate environmental heterogeneity. The loss of one of the local communities would result in changes in environmental heterogeneity and/or patch connectivity of the metacommunities (see Fig. 1, S1). The simple setting of our experiment allowed us to explicitly test the hypothesis that unique environmental conditions (i.e., being the only one local community in light or dark) and high connectivity (i.e., being the centrally located local community) define keystone communities, when considering their influences on the overall species diversity and ecosystem functioning of the metacommunity.

Materials and methods

Experimental organisms

Our experiment used seven freshwater bacterivorous ciliated protist species, including *Colpidium kleini*, *Dexiotricha granulosa*, *Paramecium bursaria*, *Paramecium caudatum*, *Spirostomum ambiguum*, *Spirostomum teres*, and *Tetrahymena thermophila*. Among these species, *P. bursaria* is the only one whose cell contains endosymbiotic green algae (*Chlorella sp.*), which supply photosynthetically produced organic carbon to their host cell and increase the amount of available organic carbon in the microcosms. The fitness of *P. bursaria* cells may thus be greater in light microcosms, relative to dark microcosms where their cells subsist only on bacteria. Prior to the experiment, we grew each of the study species separately to its carrying capacity in its stock culture.

Experimental design and setup

We used 25 mm × 150 mm Pyrex glass tubes, each of which contained 20 mL of protozoan pellet medium, as the microcosms. We first mixed the protozoan pellets (0.55 g/L; Carolina Biological Supply Company, Burlington, NC, USA) in deionized water in a 2 L flask. We sterilized the medium by autoclaving and inoculated three bacterial species: *Bacillus cereus*, *Bacillus subtilis*, and *Serratia marcescens,* as the food for the protists, into the medium. The bacterized medium was incubated at room temperature for three days prior to the experiment. At the beginning of the experiment, we added 20 mL of the medium and 0.2 mL of the stock culture of each of the seven protist species into each microcosm.

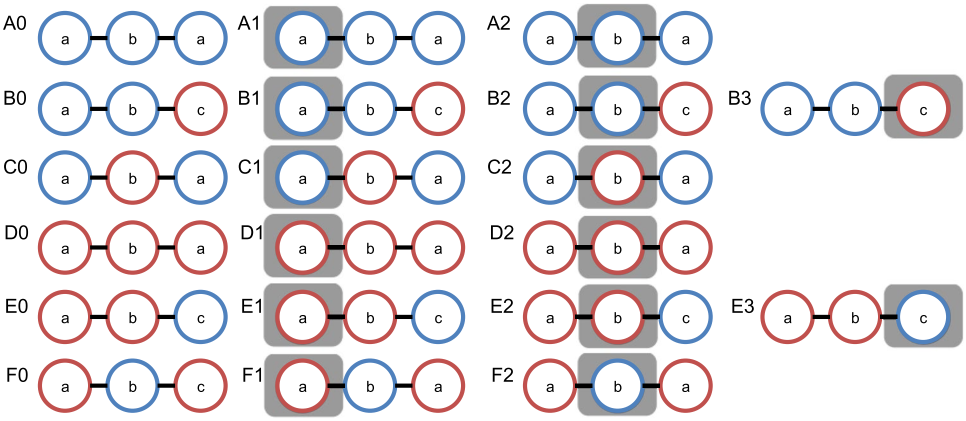

Fig. 1 Experimental design. A–F indicate different metacommunities, each of which contains three local communities. Each microcosm was assigned to either the light (red circles) or dark (blue circles) treatment. Black lines indicate dispersal among local communities. Within a metacommunity, equivalent patches are represented by the same lower-case letters. Patches in shade were removed on Week 4. The patch removal reduces the heterogeneity of metacommunities B3, C2, E3, and F2, and reduces the connectivity of metacommunities A2, B2, C2, D2, E2, and F2

The initial population size of each species was therefore set as 1% of the carrying capacity of each species. In addition, we added one autoclave-sterilized wheat seed to each microcosm as the extra carbon source. The wheat seeds were oven dried at 75 °C for 48 h and weighed before the experiment.

Our experiment included six types of metacommunities, each of which contained three microcosms that were lined up with one middle community connected to the other two local communities. We considered the protist communities in each microcosm a local community and the assemblage of the three connected microcosms a metacommunity. Under this experimental setting, we can independently control the environmental heterogeneity and connectivity in each metacommunity. We assigned each microcosm to either the light (red circles in Fig. 1) or dark (blue circles in Fig. 1) treatment to create environmental heterogeneity in the metacommunity. Metacommunities that included both light and dark patches had greater environmental heterogeneity than metacommunities that only consisted of one patch type. All possible combinations of light and dark treatments were included in the experiment (Fig. 1 A0 through F0).

We performed weekly dispersal among the three local communities in each metacommunity. We transferred 2 mL (10%) culture from each side microcosm to the middle microcosm and then transferred 2 mL culture from the middle microcosm back to each of the side microcosms. Therefore, direct culture exchange occured between the side and middle microcosms, but not between the two side microcosms. The microcosms were well mixed with a vortex mixer for 10 s before each transfer. A direct comparison of the role of discrete, weekly dispersal in our experiment to that of continuous dispersal in nature is difficult, although our dispersal rate is comparable in magnitude with the dispersal rates of zooplankton among hydraulically connected ponds (Michels et al. 2001). To estimate the population density of each protist species, we withdrew a 0.4 mL sample from each microcosm, distributed the medium into eight small drops on a petri dish, and counted the number of individuals of each species in the sample under a stereoscopic microscope. Samples containing large protist populations were diluted before counting. In addition, to replenish resources and remove the metabolic wastes, we replaced 1 mL of the culture with 1.4 mL fresh medium weekly.

All the protist populations reached equilibrium during week 4, when we removed one microcosm from each metacommunity. The loss of one of the local communities could result in the reduction in environmental heterogeneity and/or connectivity of the metacommunities. Removing the only light or dark patch would reduce the environmental heterogeneity of the metacommunity. Removing a middle patch would reduce the patch connectivity of the metacommunity, as there was no dispersal between the two side patches when the middle patches was removed. All possible ways of patch removal are shown in Fig. 1 (A1-2, B1-3, C1-2, D1-2, E1-3, F1-2), including control metacommunities in which none of the local communities were removed (Fig. 1 A0 through F0). Each combination of metacommunity type and patch removal had three replicates, resulting in 60 experimental metacommunities. After the patch removal, we ran the experiment for another three weeks and terminated it in week 7.

At the end of the experiment, the wheat seed in each microcosm was retrieved, oven dried to constant weight, and weighed. The particulate organic matter decomposition in each microcosm was quantified as the proportion of wheat seed weight loss during the experimental period. Ten individuals of each species from the experimental microcosms were randomly selected and photographed using a digital camera attached to a compound microscope. We measured their cell length and width to estimate the average cell volume of each species based on equations that approximate cell shapes (Wetzel and Likens 2000). The biovolume production in each microcosm was calculated as the sum of population biovolume of each species.

Statistical analysis

We quantified regional (γ-) and local (α-) diversity as species richness of the entire metacommunity and species richness of a single patch, respectively. To identify the keystone communities for biodiversity and ecosystem functioning, we first quantified the impacts of each local community on the metacommunity properties (e.g., biodiversity and ecosystem functions) through comparing a metacommunity's property with and without patch removal. Changes in regional species diversity were quantified as the difference in γ-diversity between a metacommunity with patch removal and that of the control without patch removal. To identify the communities that affect adjacent local communities disproportionately, we also quantified the impacts of each local community on the properties of other local communities within a metacommunity. Changes in local species diversity were quantified as the difference in α-diversity between a local community in the metacommunity with patch removal and that of its counterpart in the corresponding metacommunity without patch removal. We used similar methods to calculate the changes in two metrics of ecosystem functioning: biovolume production and particulate organic matter decomposition. Regional biovolume production and seed decomposition were calculated as the average values of local respective metrics within a metacommunity.

For a community to be keystone, it needs to have a disproportionate impact on the metacommunity properties relative to its weight (Mouquet et al. 2013). We assessed the impacts of local communities relative to their patch size, species total abundance, and biovolume, three metrics that are related to the dominance of a community and commonly used as metrics of relative weight, to ascertain whether their impacts are disproportionate (Mouquet et al. 2013). We used three-way ANOVA, followed by Tukey's HSD test, to assess the effects of heterogeneity loss, connectivity loss, and the environmental condition of the removed patch on changes in three community/ecosystem properties (i.e., species diversity, biovolume production, and seed decomposition) at both regional and local scales. We quantified the patch size as the volume of medium in each microcosm. All local patches had the same size and were identical in other characteristics, including species composition, biodiversity, and biovolume, at the beginning of the experiment. If the loss of a metacommunity feature (i.e., heterogeneity or connectivity) significantly reduces (or increases) biodiversity and/or ecosystem functions, the local communities that contribute to this metacommunity feature would have disproportionately large impacts on the metacommunity and would be considered a keystone (or burden) community for the property of interest. We also analyzed metacommunities that lost a light or dark patch separately to examine the possible influence of environmental conditions of the removed local communities on changes in community properties.

For the other two weight metrics (species total abundance and biovolume), we first regressed the impacts of local communities on their species total abundance/biovolume (Mouquet et al. 2013), acquired the standardized residuals from the regressions, and ran three-way ANOVA to test the effects of heterogeneity loss, connectivity loss, and the environmental condition of the removed patch on the standardized residuals. If the loss of a metacommunity feature (i.e., heterogeneity or connectivity) significantly reduces (or increases) the standardized residuals, the local communities that contribute to this metacommunity feature would have disproportionately large impact on the metacommunity relative to their species total abundance and biovolume, and therefore should be categorized as a keystone (or burden) community for the property of interest. All statistical analyses were conducted using R (R core team 2017).

Results

We found that the extent to which a local community contributed to spatial heterogeneity, not to connectivity, determined its contribution to the local and regional diversity of the metacommunities (Fig. 2; Table S1). At the regional scale, the removal of local communities with unique environmental conditions, which resulted in a decrease in habitat heterogeneity, significantly reduced regional diversity ($p < 0.001$; Table S1a; Fig. 2a). However, this effect significantly depended upon the environment of the removed patch ($p < 0.001$; Table S1a). Removing the only light patch significantly reduced regional-scale species diversity ($p < 0.001$; Table S1b; Fig. 2b), whereas losing the only dark patch, which also resulted in a decrease in habitat heterogeneity, did not significantly change regional species diversity ($p = 0.541$; Table S1c; Fig. 2c). The removal of the middle communities of a metacommunity, which resulted in a decrease in habitat connectivity in the metacommunity, did not affect regional diversity ($p = 0.920$; Table S1a; Fig. 2a). At the local scale, connectivity loss significantly reduced species richness (α-diversity) of the remaining local communities ($p = 0.001$; Table S1a). Both the effects of heterogeneity loss and connectivity loss on α-diversity depended on the environment of the removed patch ($p < 0.001$ and $p = 0.003$ for H*E and C*E, respectively; Table S1a). When separately analyzing the metacommunities losing a light patch and a dark patch, we found that removing a light patch contributing to either habitat heterogeneity or patch connectivity significantly reduced local species diversity ($p < 0.001$; Table S1b; Fig. 2e). On the contrary, the removal of the only dark patch that did not influence habitat connectivity of the metacommunities increased species diversity, resulting in a significant heterogeneity loss × connectivity loss term ($p = 0.023$; Table S1c; Fig. 2f). Qualitatively similar results were obtained when using total abundances and biomass as weight metrics (Table S3). These patterns were not only driven by *P. bursaria*, which was directly affected by the light and dark conditions, but also other bacterivorous species, such as *D. granulosa and P. caudatum*, whose abundance also varied among patches (Fig. S2).

Heterogeneity loss and the environment of the removed patch interactively affected regional and local ecosystem functions ($p = 0.002$, $p = 0.017$, $p < 0.001$, and $p = 0.004$ for regional biovolume production, regional seed decomposition, local biovolume production, and local seed decomposition, respectively; Table S2a; Fig. 3). Removing the only light patch significantly reduced regional and local scale biovolume production ($p = 0.007$ and $p = 0.003$ for regional and local scale biovolume production, respectively; Table S2b; Fig. 3b, e), but increased regional and local scale wheat seed decomposition ($p = 0.003$; Table S2b; Fig. 3h, k). These effects, however, were no longer significant when the effects of biovolume of the removed patch were controlled (Table S4b). Losing the only dark patch, which also resulted in a decrease in habitat heterogeneity, increased local biovolume production ($p = 0.001$; Table S2c; Fig. 3c, f) but did not significantly change regional biovolume production and wheat seed decomposition (Table S2c; Fig. 3i, l). However, neither regional nor local scale ecosystem functions were affected by the removal of the middle communities of a metacommunity (Table S1; Fig. 3a–f).

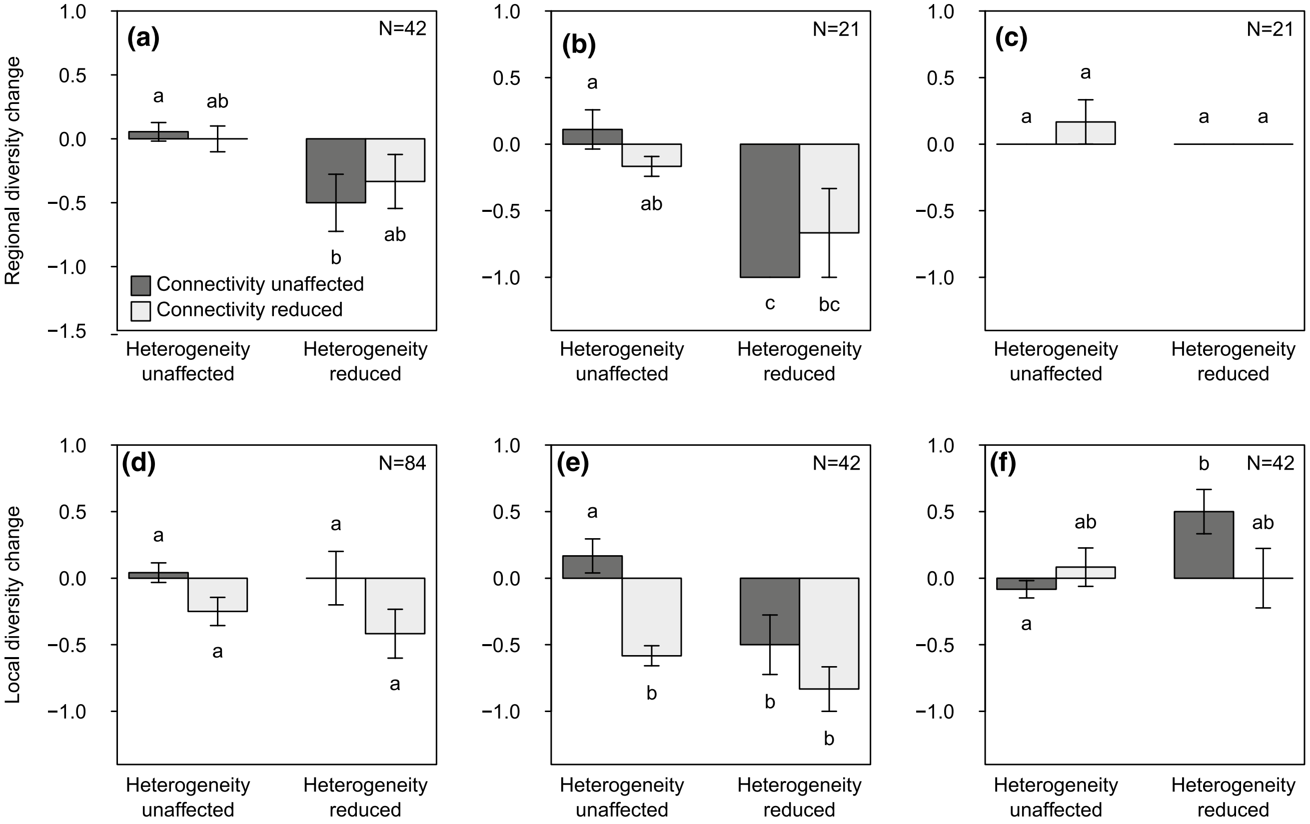

Fig. 2 Effects of heterogeneity loss and connectivity loss on changes in **a**–**c** regional and **d**–**f** local species richness. **a** and **d**, all metacommunities pooled; **b** and **e**, metacommunities losing a light patch; **c** and **f** metacommunities losing a dark patch. Values are mean + SE. Different letters indicate significant difference ($p < 0.05$) among treatments according to Tukey's HSD tests. *N* indicates sample size

Discussion

The concept of keystone communities, scaled up from the concept of keystone species, has received little attention since its inception (Economo 2011; Mouquet et al. 2013). The only empirical exception is Resetarits et al. (2018), who aimed to identify keystone communities by removing local patches at four different locations in 36-microcosm protist metacommunities. However, they found no effect of the patch removal on the biodiversity and biovolume production of the metacommunities. Our study explored whether the contributions of local communities to environmental heterogeneity and/or spatial connectivity could determine their importance in a metacommunity, and produced three novel findings. First, from the regional-scale perspective, keystone communities are those local communities containing endemic species by virtue of possessing unique environmental conditions. Removing such communities significantly reduced regional-scale biodiversity. Second, from the local-scale perspective, local communities that harbored endemic species and contributed to either environmental heterogeneity or patch connectivity had large impacts on other local communities within a metacommunity. Removing such communities significantly reduced species diversity in adjacent local communities. Third, removing keystone communities significantly altered ecosystem functions at both local and regional scales. However, the patches that were keystone for certain ecosystem functions could be a burden for other functions. We discuss these results in detail in the following paragraphs.

Theory suggests that a local community with unique environmental conditions and therefore, different species composition from other local communities, would constitute the keystone community to the metacommunity (Mouquet et al. 2013). Such keystone communities could support species that otherwise would not survive in other local communities (i.e., endemic species), making it critical for the species to persist regionally. In line with this theory, we found that the removal of the only light patch caused the largest decline in both regional diversity (Fig. 2b, S1b) and the local diversity of the remaining patches (Fig. 2e, S1c). Although the abundance of several bacterivorous species also varied among patches, this pattern is mainly associated with the fact that *P. bursaria*, characterized by a mutualistic relationship with its symbiotic green algae, coexisted with other species under light conditions but not under dark conditions.

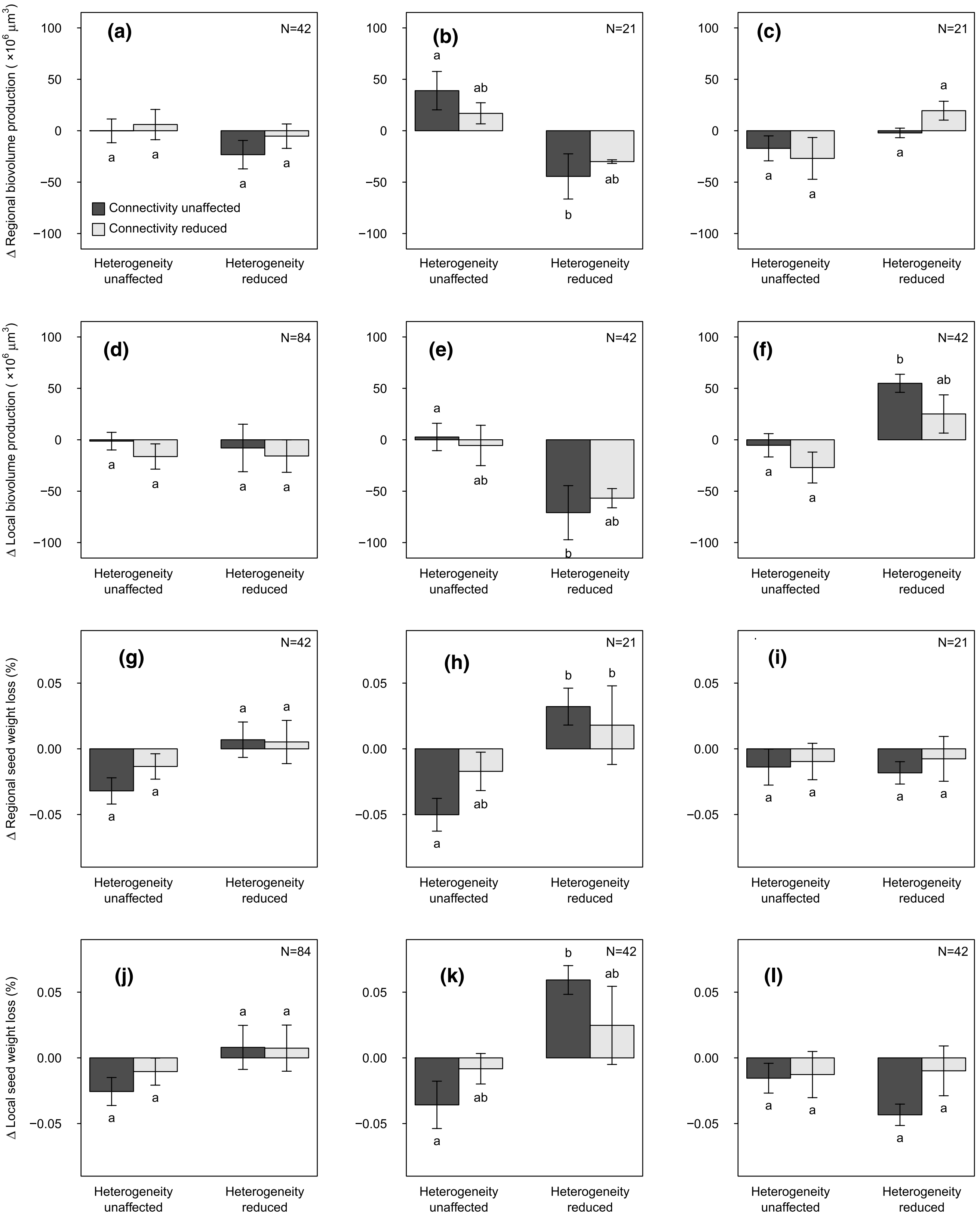

Fig. 3 Effects of heterogeneity loss and connectivity loss on changes in regional (**a–c**) and local (**d–f**) average biovolume production, and on changes in regional (**g–i**) and local (**j–l**) wheat seed decomposition of metacommunities. **a**, **d**, **g**, **j**, all metacommunities pooled; **b**, **e**, **h**, **k**, metacommunities losing a light patch; **c**, **f**, **i**, **l** metacommunities losing a dark patch. Values are mean + SE. Different letters indicate significant difference ($p < 0.1$) among treatments according to Tukey's HSD tests. *N* indicates sample size

Several observational studies have suggested the presence of keystone communities in nature. For example, temporary wetlands in an agricultural landscape in northeast Germany, featured by higher water availability than other parts of the landscape, had higher diversity of carabid beetles by favoring a group of wetland-specific beetles and supporting higher diversity in plant species (Brose 2003a, b; Tews et al. 2004). Another possible example concerns bird species in the Andaman Islands, India, where the key determinant of regional bird diversity was habitat type, particularly the presence of species-rich wet forests, the reservoirs of many habitat specialists (Davidar et al. 2001). In both examples, communities in those key habitat patches should receive high conservation priority and could be considered keystone communities; further work is nevertheless needed to demonstrate that their effects on regional biodiversity is disproportionately important. However, counterexamples have been predicted by models with neutral assumptions (Economo 2011; Gascuel et al. 2016). Economo (2011) used neutral metacommunity models to show that removing patches with high complementarity (i.e., patches that are composed of more endemic species and contribute more to gamma diversity) has no effect on diversity in a metacommunity that is driven solely by spatial structure. Our results suggest that, for non-neutral metacommunities influenced by both patch quality and spatial structure, which are more likely to be common in nature, patches with unique environmental conditions that harbor endemic species are more important than those with high similarity to other patches in maintaining biodiversity. We also found evidence for burden communities in our experiment. The removal of the only dark patch from a light-dominant metacommunity significantly enhanced species diversity of the remaining light patch, as *P. caudatum* was absent from some light patches before patch removal but was able to persist in all light patches after the dark patch was removed. This result emphasizes the importance of improving or restoring environment in low-diversity patches for conserving regional diversity due to their negative impact on the diversity of neighboring patches (Rey Benayas et al. 2009; Palmer et al. 2016).

Contribution to connectivity is another potentially important feature that influences the importance of local communities in a metacommunity, as manifested by both theoretical (Muneepeerakul et al. 2008) and experimental (Carrara et al. 2012, 2014) studies reporting that dispersal constrained by habitat connectivity can be a major determinant of the observed diversity patterns in metacommunities. However, in our experiment, the removal of connectivity had strong negative effects on the local-scale species diversity only when removing a light patch (Fig. 2e), where dark patches that lost their connection to light patches showed reduced species diversity due to the loss of source-sink dynamics. In contrast, removing a middle dark patch did not reduce species diversity in the remaining patches. Similar patterns were found in the study of the range expansion of black woodpeckers in forest patches in Catalonia (Saura et al. 2014). In that study, the amount of habitat that the black woodpecker could reach was primarily determined by the presence of stepping-stone patches with high resource availability; stepping-stones with scarce resources, however, did not promote the dispersal of the black woodpecker. We note that the patches providing connectivity did not contribute much to the maintenance of the regional-scale diversity in our experiment. This result does not mean that these stepping-stone patches are not important in the conservation of regional biodiversity, because local extinction and lack of dispersal would reduce the rescue effect and colonization rate, and may eventually lead to regional extinction (Fahrig 2002). This regional-scale impact of losing a local community may become more apparent with time, a phenomenon known as extinction debt (Tilman et al. 1994; Jackson and Sax 2010).

Biovolume production at local and regional scales declined after removing the only light patch, a pattern similar to that of regional biodiversity. *Chlorella*, the endosymbiotic green algae with *P. bursaria*, grew in the light patches, providing additional source of organic matter for the protist to subsist on. The only light patch in a metacommunity, though possessing lower biovolume compared with dark patches (Fig. S3), was featured by higher availability of organic matter over dark patches. The presence of these patches could fertilize other patches through weekly dispersal, and thus qualify as keystone for regional and local biovolume production. Similar effects of autotroph communities subsiding the biomass of connected communities were also reported in another protist microcosm experiment (Gounand et al. 2017). However, the patches that were keystone for biovolume production were burden for seed decomposition in our experiment, as removing the only light patch in a metacommunity increased seed decomposition rate at both local and regional scales. One possible explanation is that the removal of the light patches reduced the abundance of the photosynthetic *P. bursaria* and shifted the ecosystems to be more heterotrophic. Our results indicate that different ecosystem functions might not yield similar keystone communities. A patch that serves as a keystone for one function could be trivial or even a burden for another function (Mouquet et al. 2013).

We acknowledge two limitations in our experiment. First, our experimental setup, which include metacommunities consisting of three local patches and no more than two environmental conditions, was relatively simple. As such, removing a rare habitat type would cause a metacommunity to lose an entire habitat type. While this experimental design facilitated the detection of keystone communities, we recognize that losing an entire habitat type may not frequently occur in natural systems. Future studies should employ more complex metacommunity settings with more gradual heterogeneity gradients to evaluate the robustness of our results. Second, in our species pool, only one protist species *P. bursaria,* which hosts symbiont zoochlorella, responded directly to the changes in the dark and light conditions. The presence of *P. bursaria* under the light condition could increase the amount of available organic carbon in the microcosms, which would further promote the growth of bacteria and other bacterivorous protists. As a result, communities in the light patches, which supported large *P. bursaria* populations, served to be the keystone community in a metacommunity where other communities were in dark patches. Note that here our experimental test of the keystone community concept focused on the regional consequences of local community removal, which differs from keystone species studies that generally focused on the impact of losing a keystone species on a local community. It would be important, however, for future studies to explore and identify keystone communities that may not be associated with such keystone species. For example, it would be worthwhile to investigate the roles of patch size and spatial configuration in determining the importance of local communities for their metacommunity, especially in systems where environmental heterogeneity among patches is not as high as in our experiment. In addition, given the prevalence of intraspecific variation in species traits (Violle et al. 2012), future explorations of keystone communities may also need to consider species' trait variation under different environments to better understand species-level mechanisms underlying community-level responses.

Our study provided rare empirical evidence for keystone communities. For the maintenance of regional biodiversity, keystone communities are those local communities containing endemic species by virtue of possessing unique environmental conditions. As for local biodiversity, local communities play a keystone role when they contribute significantly to either environmental heterogeneity or patch connectivity. Identifying these keystone communities has important implications for effectively allocating conservation resources, given that current conservation efforts are far from sufficient to protect all species and ecosystem functions in patchy or fragmented landscapes (Pimm et al. 2014). However, there is no uniform standard of keystone community for ecosystem functions, because different ecosystem functions may yield different keystone communities. It is, therefore, more challenging to identify keystone communities to safeguard different ecosystem functions and services in conservation practices.

Author contribution statement XY and JT conceived and designed the experiments. XY, JT, and KHS performed the experiments. XY analyzed the data. XY, JT, and LJ wrote the manuscript.

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Data availability Data will be available from the Dryad Digital Repository if our manuscript is accepted.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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