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RESEARCH ARTICLE

Effect of streambed substrate on macroinvertebrate biodiversity

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Abstract Macroinvertebrates are important components of stream ecosystems, and are often used as indicator species for the assessment of river ecology. Numerous studies have shown that substrate is the primary physical environmental variable affecting the taxa richness and density of macroinvertebrates. The aim of this work is to study the effects of the characteristics of streambed substrate, such as grain size, shape, and roughness, on the composition and biodiversity of macroinvertebrates. A field experiment was done on the Juma River, a second-order mountain stream in northern China. Substrata of cobbles, hewn stones, pebbles, coarse sand, and fine sand were used to replace the original gravel and sand bed in a stretch of 30 m in length. The sampling results indicated that the macroinvertebrate assemblage is significantly affected by the grain size, porosity and interstitial dimension of the substrate, while it is rarely affected by the shape and the surface roughness of the experimental substrata. Macroinvertebrate compositions in cobbles and hewn stones were stable and changed least over time. The taxa richness and density of individuals in the substrata of cobbles, hewn stones, and pebbles are much higher than in those of the coarse sand and fine sand.

Keywords Macroinvertebrates, taxa richness, substrate, biodiversity

1 Introduction

Benthic macroinvertebrates are important components of river ecosystems, and act as a vital link in the food chain of aquatic biota. Many invertebrate species feed on algae and bacteria, while some shred leaves and filter particles of organic matter entering the river. Macroinvertebrates also constitute important sources of food and energy for larger aquatic fauna, such as fish. Because of their abundance and crucial position as 'middlemen' in the aquatic food chain, macroinvertebrates play a critical role in the natural flow of energy and nutrients in the aquatic ecosystem. Macroinvertebrates are widely used as indicator species for rapid stream ecological health assessment and biomonitoring [1–4]. Extensive research on stream macroinvertebrates can help us to better understand the river ecological processes. Knowledge of the interrelationship of aquatic fauna and environmental variables of the river ecosystem can provide guidance information for river management and river ecology restoration.

Among the physical determinants of the stream macroinvertebrate community, the substrate is the primary one and gives the best discrimination of the community [5–10]. The substrate plays the role of the principal habitat of the benthic invertebrates, and many benthic taxa exhibit different preferences for substrate [11]. The substrate characteristics which most strongly influence the resident macroinvertebrate community include the streambed grain size, heterogeneity, compactness, and stability. Previous studies in China have given more attention to the relationship between water quality and benthic communities, but have ignored the effects of the physical environmental factors, such as substrate, on the richness and biodiversity of the macroinvertebrate assemblages.

This study concentrates on the question of how different substrate characteristics, including grain size, shape, and surface roughness, affect the structure and biodiversity of the macroinvertebrates. This work seeks to address this question based on a field experiment done to understand the effect of the substrate on the macroinvertebrates better. Five types of natural mineral particles were selected to replace the original streambed substrate to study the effect of the substrate on the macroinvertebrates, while other environmental factors have the same conditions.

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2 Materials and methods

2.1 Experiment

The experiment was done in the Juma River (N39°38.4′, E115°33.2′), a second-order stream located southwest of Beijing, China. The Juma River basin has a semi-humid, temperate climate, with wet-hot summers, dry-cold winters, and short spring and autumn transitional periods. The stream drains a catchment that mainly consists of agricultural and residential areas. The experimental area was constructed along a relatively pristine stretch of 30 m in length with an average depth <0.5 m, and an average flow velocity at approximate 0.3 m/s. The macrophytes were cleared, and the original streambed substrata were dug out from the experimental stretch preceding the selected natural substrata replacement. After that, five types of natural mineral materials were placed on the experimental streambed with 3 m in length and 3 m in width, respectively, at intervals of 1.0 m (fine sand with $D_m = 0.2$ mm, coarse sand with $D_m = 1.5$ mm, pebbles with $D_m = 20$ mm, coarse hewn stones with irregular shape $D_m = 150$ mm, cobbles with $D_m = 200$ mm, where D_m is the median diameter, as shown in Fig. 1). The experimental substrata were arranged from coarseness to fineness in the direction of flow according to the grain sizes (Fig. 2), in which fine sand, coarse sand, and pebbles were laid at a thickness of 10 cm, and both cobbles and hewn stones were randomly deposited in one to two layers.

After the field construction, macroinvertebrates were allowed to colonize the experimental substrata naturally for 14 d. Macroinvertebrate samples were taken from the substrata after the colonization period at a two-week interval using a kick-net sampler (mesh width: 500 μm), and this was conducted twice. The samples were rinsed through a stainless steel sieve (mesh size: 420 μm). Then, all living organisms were sorted and picked out of each sample by hand, and were fixed in plastic sample containers with 10% formaldehyde for further analysis. In the meantime, the physical parameters were measured and recorded *in situ*, including the dissolved oxygen, water temperature, flow velocity, and water depth. In the laboratory, the macroinvertebrates in each sample were classified, counted, and identified under a stereoscopic microscope, then preserved in 75% ethanol. The taxonomic level of most individuals were identified to family or genus, and early-instar insects were only identified to higher taxonomic levels on the basis of some related references [12–15]. The total biomass of each sample was measured using an electronic balance with a precision of 0.0001 g.

2.2 Data analysis

The composition and biodiversity of macroinvertebrates collected in each substrate were evaluated via the following five biological indices:

(1) Taxa richness, S, which is the number of taxa in the samples collected, is a widely-used measure of biodiversity.

(2) Density and Biomass, which are the total number and weight of benthic organisms per unit area, are other two common indices used in evaluating benthic communities.

(3) Shannon-Wiener diversity index [16]

$$H' = -\sum_{i=1}^{S} (n_i/N)\ln(n_i/N). \tag{1}$$

where N is the total numbers of individual specimens in each sample, and n_i is the numbers of individuals in the ith taxa.

It integrates both richness and evenness, and is a commonly used diversity index.

(4) Modified Shannon-Wiener diversity index [17]

$$B = -\ln N \sum_{i=1}^{S} (n_i/N)\ln(n_i/N). \tag{2}$$

It is an index integrating richness, evenness, and total abundance.

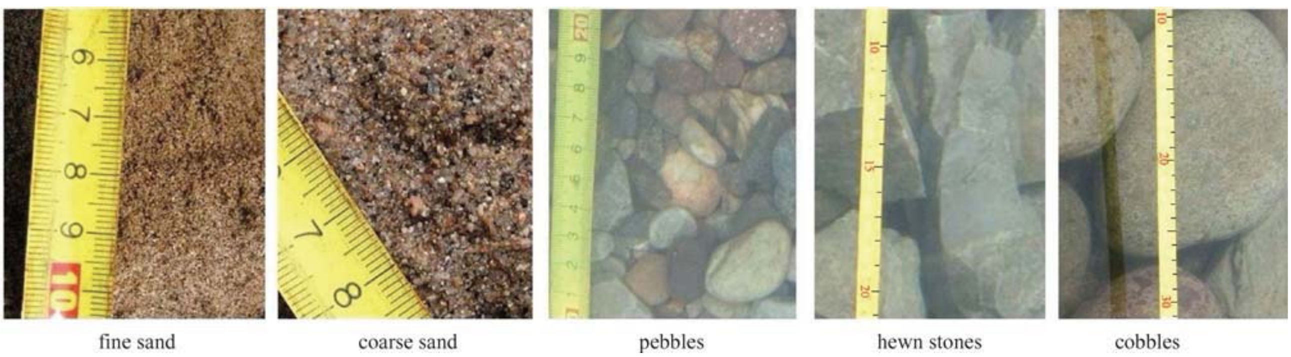

Fig. 1 Experimental mineral materials used to replace the original streambed substrate

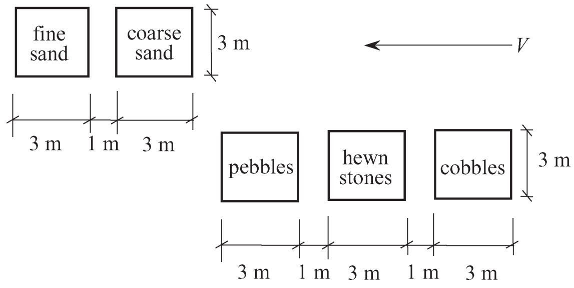

Fig. 2 Scheme of experimental area arrangement

(5) Margalef richness index [18]

$$d_M = (S-1)/\ln N. \quad (3)$$

It gives the degree of taxa richness of the bio-community.

The macroinvertebrate compositions in the experimental substrata were clustered applying the SPSS13.0 software to detect the differences of the macroinvertebrates in different substrata. Prior to clustering analysis, those taxa comprising <1% of the community in all samples were excluded, but those comprising >3% in any sample were used in the cluster analysis to minimize the effect of opportunistic species on the community. The taxa richness of macroinvertebrates was transformed by $\lg(x+1)$ to reduce the effect of the weight of dominant species on the entire benthic community. The 2 independent samples of non-parametric test results were generated using the Mann-Whitney U test in the SPSS statistical software package to test the differences of the taxa compositions per unit area among the macroinvertebrate communities in the experimental substrata.

3 Results

3.1 Density and taxa richness of macroinvertebrates

Table 1 lists the biological indices for macroinvertebrate communities in the different experimental substrata of the two samplings. Density in fine sand increased by +234.9% in the second sampling compared with that in the first sampling, while densities in the other four substrata were all reduced, in which the net reduction in hewn stones (−33.3%) and in cobbles (−34.6%) were less than that in pebbles (−49.3%) and in coarse sand (−67.8%). Taxa richness in both cobbles and hewn stones changed least over time (Table 1), indicating that the composition of macroinvertebrates is relatively stable in such substrata with large grain sizes. The average densities of two samplings in experimental substrata are ordered as follows: pebbles, hewn stones, cobbles, coarse sand, and fine sand, in which there are little differences among the substrata of cobbles, pebbles, and hewn stones.

Poole (1974) [19] considered taxa richness as the only significant biodiversity index. Generally, the larger is the taxa richness, the higher is the biodiversity. The taxa richness for each substrate can be represented by the number of taxa collected in the substrate in the two samplings, and the comparison of taxa richness in the different experimental substrata is shown in Fig. 3. Biodiversity indices of Shannon-Weiner, H', and modified Shannon-Weiner, B, for cobbles and hewn stones are both low in the second sampling due to the absolute dominance of Heptageniidae larva. However, considering the taxa richness and density of individuals, the biodiversities in pebbles, cobbles, and hewn stones are definitely larger than that in the coarse sand and fine sand (Fig. 3). Integrating the bio-assessment indices used in the analysis, the phenomenon that biodiversity is a function of substrate grain size can be observed, *i.e.* the taxa richness and the biodiversity are highest in pebbles, higher in cobbles and hewn stones, relatively lower in coarse sand, and lowest in fine sand.

3.2 Composition of macroinvertebrates

From the relative densities of the different taxa in each sample for the two samplings, it can be found that the compositions of macroinvertebrates in cobbles and hewn stones exhibit a similar variation over time (Fig. 4). The relative density of Gastropoda decreased by about two thirds in both cobbles and hewn stones with a reduction of 68.8 and 61.1%, respectively, and that of Ephemeroptera larvae in these two substrata both

Table 1 Biological indices for macroinvertebrates in different experimental substrata of the two samplings

	substrate	density/ ind·m^{-2}	biomass/ g·m^{-2}	S	H'	B	d_M
the first sampling	cobbles	752	16.4262	22	2.37	14.08	3.54
	hewn stones	800	21.4970	20	2.13	12.77	3.17
	pebbles	900	52.2319	29	2.26	15.37	4.12
	coarse sand	898	46.8584	18	1.86	11.34	2.78
	fine sand	86	18.2010	6	1.41	5.30	1.33
the second sampling	cobbles	492	5.4765	20	1.21	7.47	3.07
	hewn stones	534	4.3862	21	1.70	10.65	3.18
	pebbles	455	15.8456	24	2.40	14.67	3.76
	coarse sand	289	11.7943	11	1.57	8.89	1.76
	fine sand	288	25.4727	9	1.42	8.03	1.41

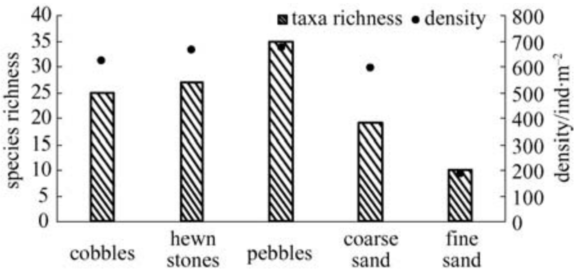

Fig. 3 Taxa richness and individuals density in experimental substrata (average of two samplings)

doubled, increasing by 98.8% and 101.1%, respectively. In cobbles and hewn stones, for the first sampling, the predominant taxa are Gastropoda which accounts for around half of the total individuals in both substrata. Ephemeroptera larvae is the second dominant, and the relative density of each taxa except of Gastropoda and Ephemeroptera are basically equal in both substrata. For the second sampling, Ephemeroptera took the status of primary dominance in cobbles and hewn stones. Gastropoda reduced to the second dominant taxa, and the relative density of the other taxa all changed a little in both substrata. When the measurement of the Euclidean distance equals to five in the clustering dendrogram chart (Fig. 5), macroinvertebrate compositions in the experimental substrata can be classified as three groups for both the first sampling and the second sampling. Cobbles and hewn stones are clustered in the same groups in both cases, which shows that macroinvertebrate structures in the both substrata have a large similarity.

Ephemeroptera, Gastropoda, and Corbiculidae are the principal dominant populations in the coarse sand as well as in the fine sand (Figs. 6a and 6b). However, the relative density of Ephemeroptera larvae is lower than that of Gastropda and Corbidulidae in the samples collected from the fine sand, and also lower than that of Ephemeroptera larvae in the samples collected from the coarse sand. The relative density of aquatic insects, excluding Ephemeroptera larvae, is more than 10% in coarse sand for the two samplings, while lower than 2.5% in fine sand. Low taxa richness and individual density in fine sand are associated with its small grain sizes, which provides fewer suitable living spaces for macroinvertebrates to inhabit. Aquatic insects have large difficulty to survive there, and mollusks became the predominant taxa. Thus, fine sand is inhospitable to invertebrates, and it simplifies the composition of macroinvertebrates. Ephemeroptera and Gastropoda are the dominant taxa in the pebbles, which is the same as for the other four substrata. The difference is that the macroinvertebrate community in the pebbles has a larger evenness and diversified taxa composition.

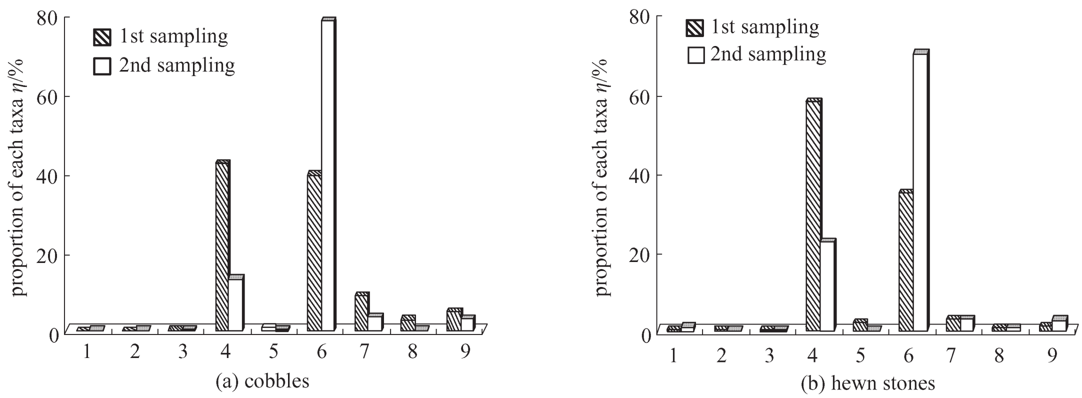

Fig. 4 Variation of macroinvertebrate compositions in the cobbles and hewn stones
1. Oligochaeta, 2. Hirudinea, 3. Turbellaria, 4. Gastropoda, 5. Lamellibranchia, 6. Ephemeroptera, 7. Diptera, 8. Trichoptera, 9. Other taxa

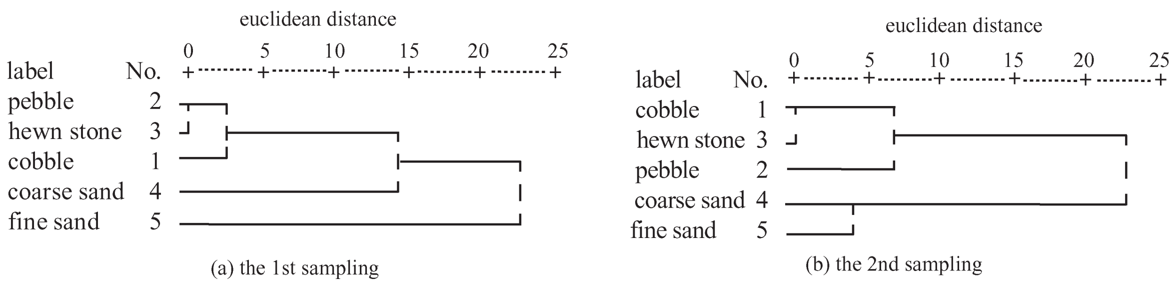

Fig. 5 Clustering dendrogram of taxa collected from the experimental substrata for two samplings

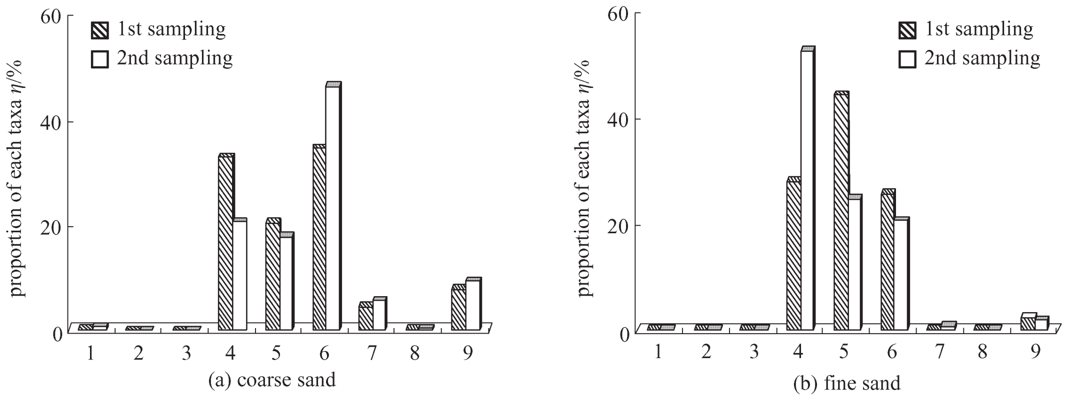

Fig. 6 Variation of macroinvertebrate compositions in the coarse sand and fine sand
1. Oligochaeta, 2. Hirudinea, 3. Turbellaria, 4. Gastropoda, 5. Lamellibranchia, 6. Ephemeroptera, 7. Diptera, 8. Trichoptera, 9. Other taxa

Results of the nonparametric test show that macroinvertebrate compositions in the pebbles, cobbles and hewn stones exhibit little differences ($P > 0.05$) in the two samplings. The difference of the invertebrate compositions between in the coarse sand and in the pebbles changed from non-significance ($P > 0.05$) for the first sampling to significance ($P < 0.05$) for the second sampling, and the difference between the fine sand and the substrata of cobbles, pebbles, and hewn stones changed from obvious significance ($P < 0.01$) to significance ($P < 0.05$). The difference of bio-communities between the fine sand and the coarse sand changed from significance ($P < 0.05$) to non-significance ($P > 0.05$). The diversified benthic composition in the pebbles substrate, due to the preferences of many benthic groups for it, enlarges the difference from the benthic community in the coarse sand. Epiphytic mosses on the substrate increase the opportunity of benthic species colonizing the fine sand, which reduces the difference of benthic communities between the fine sand and the coarse sand. The smallest difference of invertebrate communities between cobbles and hewn stones gives more evidence that a large similarity of benthic communities exists between both substrata.

4 Discussion

4.1 Effect of bed material grain size on macroinvertebrates

Bed material grain size has a significant effect on the macroinvertebrate community, and the compositions and dominant taxa of benthic community differ greatly between the substrata with different grain sizes. The results of this study show that the biodiversity is highest in pebbles, higher in cobbles and hewn stones, and lower in coarse sand and fine sand. Sandy substrate, where fine sand is the worst substrate for macroinvertebrates, is inhospitable to benthic fauna, and the benthic community within it has the lowest taxa richness. The substrata with large grain sizes, such as cobbles and hewn stones, are stable and can protect the benthic organisms from environmental disturbance, yielding the result that the structure of macroinvertebrates in these two types of experimental substrata changed least over time. Reice (1985) [20] also found that the structure of benthic macroinvertebrates has the largest resilience in cobbles because the large quantities of interstitial spaces in cobbles provide stable and diversified habitats for invertebrates to colonize and use for refuge.

4.2 Effects of interstice dimension and porosity of substrate on macroinvertebrates

Benthic macroinvertebrate community in the pebbles has larger biodiversity than that in the cobbles for two samplings, which is consistent with the result of Alexander and Allan (1984) [9] who found that invertebrates were more abundant in loose gravel/pebbles compared with that in loose cobbles. Both pebbles and cobbles have large interstice dimensions, and thus can provide large living spaces for invertebrates to inhabit. However, the interstitial spaces in pebbles occupy a larger proportion than that in cobbles, while their volumes are basically equal to each other, and thus there are more diversified taxa living in pebbles based on the hypothesis that diversified habitats/niches support diversified communities. The biodiversity of macroinvertebrates is positively correlated with both interstice dimension and porosity of substrate, and a function of them can be expressed in the form of $D = f(R, e, E_{vi})$, in which D is the biodiversity, R is the interstice dimension, e is the porosity, and E_{vi} is the other environmental factor. The larger the interstitial spaces of the bed material are, the more hospitable is the substrate to invertebrates, and if the interstice spaces are favourable and the porosity is larger, the substrate is more hospitable to invertebrates. The large heterogeneity of the substrate resulting from the interaction effect of interstice dimension and porosity can support large biodiversity. Generally, porosity has a negative relationship with the median diameter of the bed material, while interstice dimension has a positive relationship with it. The pebble substrate integrates the advantages of relatively suitable interstice dimension and high porosity. Thus, the biodiversity of the bio-community is larger in pebbles than in the other types of substrate.

4.3 Effects of shape and surface roughness on macroinvertebrates

Results of clustering analysis and non-parametric testing show that invertebrate communities in both substrata of cobbles with smooth surface and irregular hewn stones with rough surface exhibit a similar variation over time. Previous studies give inconsistent conclusions about the effect of substrate surface texture on invertebrates. Erman and Erman (1984) [8] found that rocks with high surface heterogeneity (roughness) were colonized by a larger number of individuals (but not taxa) than rocks with low surface heterogeneity. While Downes *et al.* (2000) [21] found that surface texture had no effect on density, but can increase the species richness. This study finds that invertebrate density in hewn stones is just a little larger than that in cobbles, and the taxa richness in these two substrata have little difference. Thus, the surface roughness and the external shape of the substrata in the experiment have little effect on the composition and the individual species density of the macroinvertebrates, and no effect on taxa richness. The cobble substrate selected in the experiment has some pits or cracks, and thus the surface roughness of it has no significant difference from that of the hewn stones, resulting in less distinct differences in roughness than in previous studies.

5 Conclusions

Grain size, porosity, and interstice dimension of the substrate all have significant effects on the composition of macroinvertebrates. The biodiversity is the highest in pebbles, higher in cobbles and hewn stones, relatively low in coarse sand, and the lowest in fine sand. The macroinvertebrate structure and the dominant taxa differ obviously in substrata with different grain sizes. The composition and the diversity of the benthic invertebrates are positively correlated with both interstice dimension and porosity of the substrate. A substrate with large interstitial spaces is favourable to invertebrates, and that with large porosity is hospitable to invertebrates if the interstitial space is suitable. The higher the heterogeneity created by the interaction of the interstitial space and porosity, the more diversified is the macroinvertebrate composition. The compositions of the macroinvertebrates between cobbles and hewn stones have a high similarity and exhibit a similar variation over time, which indicates that shape and surface roughness of the experimental substrata have little effect on the structure and density of the benthic community, and no effect on the taxa richness. Macroinvertebrate compositions are stable in cobbles and hewn stones, and thus the substrata with large grain sizes can protect macroinvertebrates from environmental disturbance.

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