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Land Consolidation with Seedling Cultivation Could Decrease Soil Microbial PLFA Diversity

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ABSTRACT

The impact of land consolidation on the soil microbial PLFA diversity is of great importance for understanding the effective arable land usage, improving agricultural ecological conditions and environment. In this study, we collected the soil samples (0–20 cm) in experimental plots with 0 (Z_0), 1 (Z_{1a}) and 4 (Z_{4a}) years of land consolidation in the forest station of Ningbo City, Zhejiang Province, southeastern China. The results were analyzed using ANOVA for randomized block design. Compared with control (Z_0), the soil pH value under Z_{1a} treatment increased by 14.6%, soil organic carbon (SOC) content decreased by 65.4%, so did the PLFA contents and relative abundance of all the microbial PLFA diversity ($P < 0.05$), respectively. Meanwhile, for the Z_{1a} treatment, the ratio of fungi to bacteria (F/B) significantly decreased by 35.9% ($P < 0.05$), while the ratio of Gram-positive bacteria to Gram-negative bacteria (G+/G-) significantly increased by 56.1%. This was strongly related to the increased soil pH values and the decrease of SOC. The Shannon index (H) and evenness index (E) of soil microbial PLFA diversity were significantly decreased after land consolidation ($P < 0.05$). Compared to the Z_1 treatment, the microbial PLFA diversity was improved slightly. Therefore, the land consolidation could significantly affect the composition of soil microbial PLFA diversity, and decrease the soil ecosystem stability.

KEYWORDS

Cultivated land balance; land use change; *Pinus taiwanensis* forest; PLFA; soil degradation; soil fertility variation

1 Introduction

Sustainable land use is of importance to the economic development of human society [1,2], especially for China, which is a developing country with a large population, but few cultivated land per capita. From 1961 to 2015, the arable land per capita of China dropped from 0.16 ha to 0.09 ha, which was consistently lower than the average level of the world (0.37 ha in 1961 and 0.19 ha in 2015) [3,4]. Since 1980s, due to the rapid socio-economic development and urbanization, a large amount of arable land was occupied [5]. China's arable land area decreased from 123 million ha to 121 million ha during the decade between 2000 and 2010 [6]. The decreasing arable land has seriously threatened the food production. By 2030, the total amount of arable land required is expected to be 146 million ha to ensure national food security [7]. Currently, urbanization is the main reason for the decrease of arable land [8]. During 1950 to 2015, the



urbanization rate increased rapidly from 11.18% to 56.1%, leading to the shrinkage of arable land resources [7,9]. In addition, inefficient use of construction land, scattered and fragmented farmland, serious soil pollution and land degradation have caused serious food security and ecological issues. In order to deal with the increasing demand for arable lands, land consolidation is regarded as an effective approach.

Land consolidation adopts various measures to promote the quality of arable land, increase the effective arable land area, and improve agricultural ecological conditions and environment [2,10–13]. At present, land consolidation has been widely used [14,15]. From 2001 to 2015, China has replenished arable lands of 4.52 million ha through land consolidation [16].

Due to the different natural condition, land consolidation measures and management methods, the results obtained are quite different or opposite. After land consolidation, soil available phosphorus (AP), soil available potassium (AK) and soil organic carbon (SOC) decreased significantly, while pH value increased significantly [17]. The soil bulk density increased by 1.34 times after land consolidation [18]. Since the shrub coverage in woodlands has a significant positive correlation with soil organic carbon density (SOCD) [19], the cultivated land deriving from natural forest could decrease both the SOC and soil total nitrogen (TN) [20,21]. After land consolidation, the total value of ecological services in different regions decreased by 10.5%~33.1% [22], the fragmentation of arable land landscape increased [23], and the biological fertility index (BFI) decreased [21]. While other study revealed that land consolidation improved the soil quality [24]. Meanwhile, previous researches focused on the soil physical and chemical properties, the impact of land consolidation on soil microorganisms was rarely reported.

Soil microbial community is one of the most important functional components in soil biota, and it is a sensitive indicator of soil ecosystem changes, indicating changes in soil quality [25]. In general, different soil microorganisms can control the decomposition of multiple substances and the circulation of different nutrients in the soils [26]. Changes in soil quality caused by land consolidation are not only reflected in the negative impact on the content of SOC and labile carbon pools [27], but also reflected the changes in the amount of soil microorganisms [28–30]. However, there are few studies on the change of soil microbial community after land consolidation from forest land to arable land. At present, phospholipid fatty acid (PLFA) analysis is widely used in the study of soil microbial community diversity [31]. By analyzing soil microbial PLFA, microbial biomass, the total biomass and microbial community structure of different groups can be quantitatively reflected [32]. The objective of this study was to: investigate the variation of soil microbial PLFA diversity under different land consolidation years, in order to reveal the significant effect on soil microbial PLFA diversity caused by land consolidation and its impact on soil quality.

2 Material and Methods

2.1 Study Site and Sample Collection

The study was carried out at the forest station of Ningbo City, Zhejiang Province, southeastern China (29°38'–29°48' N, 121°13'–121°58' E). This area has a typical subtropical monsoon climate. The annual precipitation is approximate 2000 mm. The annual daylight hours and frost-free days are 1813 h and 240 d, respectively. The soil is classified as Ferralsols in the Food and Agriculture Organization (FAO) soil classification system.

In this study, land consolidation referred to the lands which were derived from the originally natural forests and further used as arable lands for seedling cultivation in southern China, due to lacking arable lands in this region. The experimental plots with 1 (Z_{1a}) and 4 (Z_{4a}) years of land consolidation period and reference plot (Z_0) were selected from four watersheds of 20, 25, 26, 18 ha. Z_0 treatment stands for the *Pinus taiwanensis* forest as reference plots. During 2013 and 2016, the *Pinus taiwanensis* forest land was used for arable land. The above hillside fields were transformed into terraced fields (not shown), with strong soil disturbance. The Z_{1a} plots were set up in 2016 after land consolidation and no plants were in

these plots. The Z_{4a} plots (for seedling cultivation) had a land consolidation history since 2013, and arbor seedlings (*Taxus wallichiana* var. *mairei*, *Chamaecyparis obtusa*, etc.), has been planted, with an average seedling height of 0.8 m, an average ground diameter of 2.0 cm and a density of 30,000 plants per ha. The annual application of compound fertilizer (N:P₂O₅:K₂O = 15:15:15) was 600 kg ha⁻¹ for Z_{4a} plots. Altogether, 12 plots were selected in the watersheds that served as 4 blocks. The slope, gradient and soil type of the plots in the same block were similar.

Soil samples (0–20 cm) were taken from the study plots in October, 2017. Six sub-samples from the same plot were mixed thoroughly to form a composite sample. The soil samples were preserved on ice for 3 hours before being shipped to the laboratory [33]. Soil samples were passed through a 2-mm nylon mesh. Part of the samples were used for the analysis of microbial community structure. Another half of samples were air-dried and used for determination of physical and chemical properties of the soil [33].

2.2 Soil Chemical Analysis

Soil pH was measured with a pH meter using an aqueous suspension (soil-water ratio of 1:2, W:V). The soil organic carbon content was determined using H₂SO₄ and K₂Cr₂O₇, and titrating with Fe (NH₄)₂·(SO₄)₂·6H₂O solution. Alkali-hydrolyzable nitrogen was analyzed using alkali-diffusion method. Available phosphorus and potassium were determined by the NaHCO₃ extraction-colorimetry and NH₄OAc extraction-flame photometry methods, respectively [34].

The extraction and analysis of PLFAs were carried out as described by Frostegård et al. [35,36]. In this study, the relative abundance of the PLFA was expressed in mol %. The communities of Gram+ bacteria were indicated by PLFA i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0. Gram- bacteria were represented by 16:1ω7c, cy17:0, 18:1ω7c and cy19:0 [37]. Bacteria were characterized by i14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 17:0, i17:0, a17:0, cy17:0, 18:1ω7c, cy19:0 [38]. Fungi were characterized by 18:1ω9c and 18:2ω6c [39]. Arbuscular mycorrhizal fungi were represented by 16:1ω5c [39]. Actinomycetes were characterized by Me16:0, Me17:0 and Me18:0. Protozoans were characterized by 20:4w6, 9, 12 and 15c [40].

2.3 Statistical Analysis

The analysis of variance (ANOVA) for randomized block design was applied to test the land consolidation effects on pH, SOC, AN, AP, AK, PLFA, relative abundance of PLFA, the ratio of fungi/bacteria and G+/G-. A multiple comparison using Duncan's multiple range test method was conducted when a significant effect was detected ($\alpha = 0.05$). If the data did not meet the assumptions of normality, values were log transformed prior to ANOVA analysis. Statistical analyses were carried out using SPSS[®] for windows (version 18.0). Graphing was achieved with Origin 2019 software.

3 Results

3.1 Soil Chemical Properties

After land consolidation, the soil pH values increased significantly ($P < 0.05$), while the SOC contents decreased significantly ($P < 0.05$) (Table 1). Meanwhile, the changes of AN, AP and AK were not significant during the land consolidation process (Table 1).

Table 1: Impacts of land consolidation on soil physical and chemical properties

Treatment	pH	SOC (g kg ⁻¹)	AN (mg kg ⁻¹)	AP (mg kg ⁻¹)	AK (mg kg ⁻¹)
Z ₀	4.1 ± 0.1b	26.81 ± 2.64a	60.74 ± 8.11a	0.69 ± 0.02a	63.05 ± 7.69a
Z _{1a}	4.7 ± 0.2a	9.29 ± 3.16b	48.25 ± 16.25a	0.52 ± 0.45a	52.21 ± 11.32a
Z _{4a}	4.6 ± 0.2a	9.35 ± 2.53b	54.74 ± 17.63a	1.31 ± 1.25a	64.35 ± 17.61a

Note: Different small letters in the same column indicate significant differences among different tree-age treatments ($P < 0.05$). SOC: Soil organic carbon content; AN: Alkali-hydrolyzable nitrogen; AP: Available phosphorus; AK: Available potassium.

3.2 Soil Microbial PLFAs Components

Soil bacteria PLFAs, fungi PLFAs, actinobacteria PLFAs, arbuscular mycorrhizal fungi (AMF) PLFAs and protozoan PLFAs ranked as follows: $Z_0 > Z_{4a} > Z_{1a}$ (Fig. 1). The total PLFA concentration, as an indicator of active soil microbial biomass, was highest in the Z_0 soil samples (not shown). Compared with Z_0 treatment, the bacteria PLFAs, fungi PLFAs, actinomycetes PLFAs, AMF PLFAs and protozoan PLFAs of Z_{1a} and Z_{4a} decreased significantly ($P < 0.05$), respectively (Fig. 1). While compared with Z_{1a} treatment, the PLFAs of all components of soil microbial biomass was slightly increased after 4 years of land consolidation Z_{4a} , but the differences were not significant ($P > 0.05$) (Fig. 1).

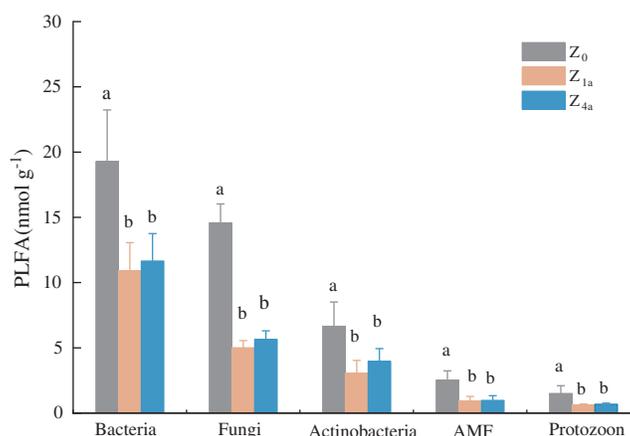


Figure 1: Soil phospholipid fatty acid contents on treatments with different consolidation history. Different letters indicate differences at $P < 0.05$ level. AMF = Arbuscular mycorrhizal fungi

The soil microbial community significantly changed after land consolidation (Figs. 2 and 3). The relative abundance analysis of PLFA showed that bacteria dominated in the soils, while the second most abundant component was the fungus and actinobacteria. Arbuscular mycorrhizal fungi and protozoans were less abundant in soil samples. Both the relative abundance of soil G⁻ and G⁺ bacteria biomass ranked as follows: $Z_0 > Z_{4a} > Z_{1a}$. Compared with Z_0 , after 1 year of land consolidation, the relative abundance of PLFA in all groups was significantly decreased ($P < 0.05$) (Fig. 2). while compared with Z_{1a} , after land consolidation for 4 years, the relative abundance of PLFA in all groups slightly increased, but the difference was still not significant ($P > 0.05$) (Fig. 2).

Compared with Z_0 , after 1 year of land consolidation, the soil fungal/bacteria ratio (F/B) decreased significantly ($P < 0.05$), and G⁺ bacteria to G⁻ bacteria significantly increased ($P < 0.05$) (Fig. 2). Compared with Z_{1a} , after land consolidation with 4 years, F/B increased, while G⁺/G⁻ decreased slightly, but the difference was not significant ($P > 0.05$) (Fig. 3).

3.3 Soil Microbial Diversity Index

Both the microbial diversity index (Shannon index, H) and evenness index (E) for the different treatments were in the following order: $Z_0 > Z_{4a} > Z_{1a}$. The H and E in Z_0 treatment was much greater than in the Z_{4a} and Z_{1a} treatments ($P < 0.05$), but there were no significant differences in H and E between the Z_{4a} and Z_{1a} treatments (Table 2).

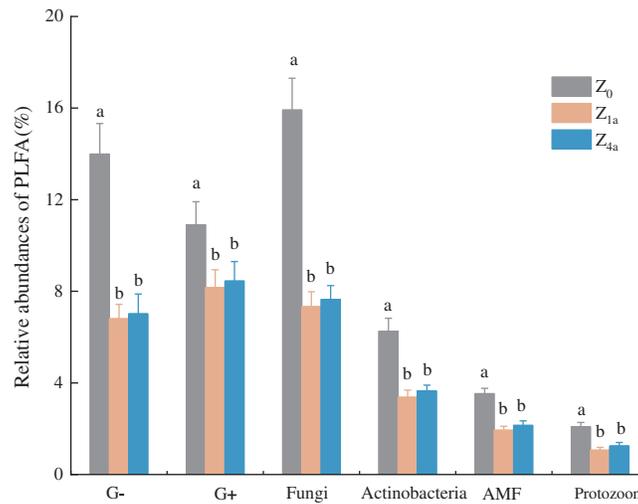


Figure 2: Relative abundance of phospholipid fatty acids of microbial groups in different consolidation history. (Different letters indicate differences at $P < 0.05$ level. G⁻ = Gram-negative bacteria; G⁺ = Gram-positive bacteria; AMF = Arbuscular mycorrhizal fungi)

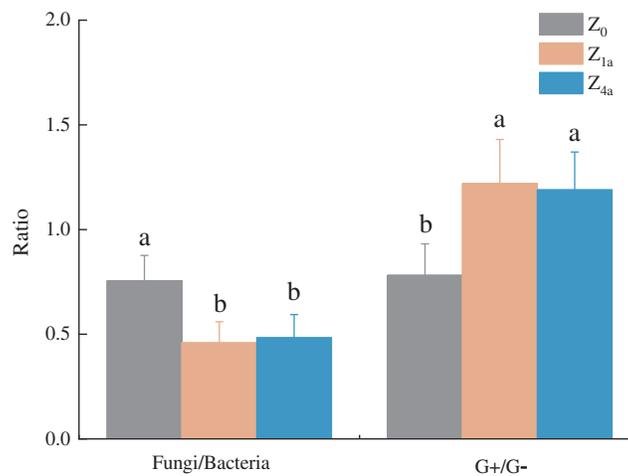


Figure 3: Ratio of Fungi/Bacteria and G⁺/G⁻ within three treatments. Different letters indicate differences at $P < 0.05$ level

Table 2: Indexes of microbial PLFA diversity in the soils

Treatment	Shannon index (H)	Evenness index (E)
Z ₀	3.735 ± 0.075a	0.979 ± 0.012a
Z _{1a}	3.216 ± 0.054b	0.928 ± 0.001b
Z _{4a}	3.358 ± 0.078b	0.946 ± 0.024b

Note: Different small letters in the same column indicate significant differences among different treatments ($P < 0.05$). Different small letters in the same column indicate significant differences among different treatments ($P < 0.05$).

3.4 Correlation between Soil Chemical Properties and Microbial PLFA Diversity

Soil protozoan, actinomycetes, arbuscular mycorrhizal fungiprotozoa and fungi were negatively and significantly correlated with soil pH ($P < 0.05$ or $P < 0.01$) (Table 3). Soil protozoa, fungi, actinomycetes and arbuscular mycorrhizal fungi were positively and significantly correlated with SOC ($P < 0.05$ or $P < 0.01$) (Table 3). Soil fungi, actinomycetes and protozoa was negatively and significantly correlated with soil available phosphorus ($P < 0.05$ or $P < 0.01$) (Table 3).

Table 3: Correlation analysis of PLFA contents and soil properties in soil

Variables	B	F	A	AMF	P
pH value	-0.509	-0.935**	-0.667*	-0.673*	-0.654*
SOC	0.589	0.919**	0.806**	0.883**	0.903*
AN	0.571	0.419	0.170	0.003	0.146
AP	-0.513	-0.670*	-0.580*	-0.570	-0.832**
AK	-0.245	-0.223	-0.154	-0.193	-0.070

Note: **, *: significant at $P < 0.01$, $P < 0.05$, respectively. SOC = Soil organic carbon content; AN = Alkali-hydrolyzable nitrogen; AP = Available phosphorus; AK = Available potassium; F = Fungi; B = Bacteria; A = Actinomycete; AMF = Arbuscular mycorrhizal Fungi; P = Protozoo.

4 Discussion

4.1 The Effect of Land Consolidation Change on SOC and pH

Land consolidation changed the underground ecosystems, resulting in destruction of biodiversity and reduction of soil organic carbon [41,42]. When manure is applied during land consolidation process, the microbial community diversity in soils are significantly higher than those areas with only chemical fertilizer application [43,44], as the application of manure into the soil can provide the carbon-containing substances and increase the soil organic matter (SOM) [45,46]. In this study, the SOC decreased significantly after land consolidation. The microbial PLFA diversity after land consolidation was closely related to the decrease of available nutrient content (SOC). This result is consistent with the previous findings that SOC is one of the main soil properties related to the changes in soil microbial community structure [47]. Meanwhile, the PLFAs of fungi, actinomycetes, arbuscular mycorrhizal hyphae (AMH) and protozoa were all positively and significantly correlated with SOC (Table 3), especially the differences in soil organic carbon often lead to the variation in microbial community composition [48]. Correlation analysis also showed that the PLFAs of different soil microorganisms were positively correlated with the available nutrients in soils [49].

In this study, the topsoil (0–15 cm) which generally had relatively high organic carbon content, was stripped during the land consolidation process, the bottom soil was exposed, and the SOC content decreased significantly (Table 1), which led to a decrease in the PLFAs components (Fig. 1). This was consistent with the finding of the study that the difference in microbial composition was related to the available carbon content in soil [50]. At the same time, soil pH increased significantly after land consolidation (Table 3), and pH was also one of the main soil characteristics that caused microbial community structure changes [47,51]. At a low pH, the content of fungi PLFAs is more abundant [52]. In our study, a negative and significant correlation between the content of fungi PLFAs and soil pH was found. Meanwhile, the contents of PLFAs of actinomycetes, arbuscular mycorrhizal hyphae (AMH), and protozoa had also a negative and significant correlation with soil pH (Table 3), which indicated that in a certain pH range, the stronger the acidity of the soil, the richer in the microbial community [53]. Therefore, changes in SOC and pH after land consolidation are the main influencing factors for changes in PLFAs of soil microbial community.

4.2 The Effect of Land Consolidation Change on Soil Microbial PLFA Diversity

Soil microbes are considered to dominate SOM decomposition and nutrient cycling in soils [26], while bacteria and fungi, account for about 90% of soil microbial biomass. Abundant SOC is a desired environment for fungal growth [54]. The composition of the soil microbial PLFA diversity changed significantly after land consolidation (Figs. 1 and 2). Since the location and site condition were almost the same in this research, land consolidation was considered to be the main factor that caused the significant differences in microbial PLFA diversity [55]. Arbuscular mycorrhizal fungi (AMF) are also an important part of the rhizosphere microbial community, and they can form mycorrhizal symbiotes with the roots of most terrestrial plants, accounting for more than 10% of the total soil microbial biomass [56,57]. AMF has a positive promotion effect on plant growth. Therefore, exploring which soil factors affect AMF is important to further reveal the changes of AMF after land consolidation. Studies have found that there is a negative correlation between soil pH and AMF abundance diversity [58]. This is consistent with the finding of this study. However, other study has found a positive correlation between soil pH and AMF diversity [59]. Soil pH is considered to be a key driver of AMF abundance diversity, but its positive or negative effect need further to be determined based on specific study area. The results of this study indicated that there was a significantly positive correlation between the content of AMF PLFAs and SOC content, which was contrary to other studies that considered SOC as the main negative driving factor for AMF development [60–62].

F/B ratio can reflect the relative content of fungi and bacteria, and the relative abundance of the two populations. Their ratio reflects the quality of SOM in the soil system. In farmland ecosystems, the higher the F/B ratio, the more stable the ecosystem [63]. After land consolidation, F/B decreased significantly (Fig. 3), indicating that land consolidation reduced the stability of the soil ecosystem. Whether before or after land consolidation, the F/B ratio was always less than 1 (Fig. 3), which did not mean that the contribution of fungi was less important than that of bacteria. In general, some substances in the soil must be decomposed by fungi before being used by bacteria. Therefore, fungi and bacteria are interdependent, and both are indispensable in the decomposition process. The PLFA method may underestimate the fungal biomass and lead to an F/B biomass ratio <1.0 [64].

Soil bacteria can be divided into two major groups, G⁺ and G⁻. G⁺ (such as *Arthrobacter*) is more tolerant to environmental stress (for example, hunger) than G⁻ (*Pseudomonas*) [65]. G⁺ such as *Arthrobacter* grows faster in an oligotrophic environment [66]. Studies have found that with the removal of existing trees and root tissues in the soil, the G⁺/G⁻ ratio increases, and ecosystem productivity decreases [67]. Consistent with the finding that the G⁺/G⁻ ratio increased significantly after land consolidation in this study. A high proportion of G⁺ is considered to be a transition from eutrophication to oligotrophic soil environment, and G⁺/G⁻ reflects changes in bacterial community structure [68]. G⁺/G⁻ elevation is closely related to SOC quality decline [69]. And G⁻ bacteria uses more plant-derived C sources, while G⁺ bacteria uses more SOM-derived carbon sources [70]. When comparing natural ecosystems, the G⁺/G⁻ ratio can be used as a rough indicator of the relative C utilization of the bacterial community [59]. After land consolidation, G⁺/G⁻ increased significantly due to the amount of SOC loss (Fig. 3). With the extension of land consolidation period and human management, various soil nutrients have increased. After 4 years of consolidation, G⁺/G⁻ decreased slightly. After land consolidation of 4a, the ratio of G⁺/G⁻ was slightly lower than that of land consolidation of 1a, probably because the planted crops increased the C source of plants, and the soil ecosystem and biodiversity were restored to a certain extent.

5 Conclusions

The content and relative abundance of PLFAs in soil significantly decreased after land consolidation, which was closely related to the significant decrease of SOC. F/B decreased significantly, while G⁺/G⁻ increased significantly, both related to the decrease of SOC and the increase of pH value. After land consolidation, both Shannon index and evenness index of soil microbial diversity decreased significantly.

Land consolidation strongly effected soil microbial PLFA diversity and its composition. This study demonstrated that land consolidation significantly affected the composition of soil microbial PLFA diversity and reduced the stability of soil ecosystems. As the land consolidation period extended, the soil microbial quality would improve.

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Authors' Contributions: The authors confirm contribution to the paper as follows: study conception and design: Wu JS, Liu J; data collection: Jin J, Yan BJ; analysis and interpretation of results: Yan BJ, Jian YQ, Liang C.; draft manuscript preparation: Zhang S. All authors reviewed the results and approved the final version of the manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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