

INVESTIGATION ON THE VEGETATION DISTRIBUTION LANDFILL COVER, METHANE OXIDATION CAPACITY OF VARIOUS RHIZOSPHERE SOIL AND RHIZOSPHERE MICROECOLOGY IN THE PROCESS OF MSW LANDFILL

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Abstract: Landfill gas pollution control has always been an important content in the field of solid waste, and the establishment of vegetation in the cover layer of municipal solid waste landfill is an important means to control disordered landfill gas escape. In this study, a systematic investigation was conducted on the vegetation of unclosed landfills under the subtropical monsoon humid climate in Chongqing, China, to investigate the methane oxidation capacity of rhizosphere soil, microecological characteristics of rhizosphere and the distribution of metabolites. It is found that more than 22 kinds of landfill gas highly tolerant vegetation grow well in the cover layer, and the vegetation community evolves to herbaceous vegetation. The results of methane oxidation capacity analysis showed that the average methane degradation capacity of each vegetation rhizosphere soil was higher than that of non-rhizosphere soil, and the methane oxidation rate of *Rumex Acetousa* rhizosphere soil was the strongest, which was 0.0800 g_{CH₄}/(kg_{soil} · h), which was nearly 20 times that of non-rhizosphere soil, and the methane oxidation efficiency of *Rumex Acetousa* rhizosphere soil was the highest, which was 90.32%. The results of diversity sequencing showed that the landfill cover soil under the action of vegetation could enrich a variety of microorganisms and effectively improve the uniformity and diversity of microbial community. *Methylobacterium* has a strong ability of methane bio-oxidation, and the synergistic effect of a variety of microorganisms and methane oxidizing bacteria can effectively promote the biotransformation of pollutants. The analysis of metabolites and pathways of rhizosphere microorganisms showed that the Arginine in root exudates could promote microbial TCA cycle, accelerate metabolic efficiency, induce plant secondary metabolites and plant hormones to regulate microbial community structure. Root exudates regulate the community structure and metabolic

characteristics of rhizosphere microorganisms by inducing specific metabolic processes of rhizosphere microorganisms, so as to obtain higher methane bio-oxidation ability in rhizosphere soil. The results show that the extensive planting of *Rumex Acetousa* and other vegetation in the landfill cover is of great significance to control the disordered emission of landfill gas.

Keywords: Landfill cover, Vegetative root system, Bio-oxidative activity, Functional microorganisms, Metabolic pathways

1 INTRODUCTION

Sanitary landfill is one of the main treatment methods of solid waste. During the stabilization operation of landfill waste, complex biochemical reactions take place and a large amount of landfill gas is produced. The main components are methane CH₄ (55 vol% 60 vol%), carbon dioxide CO₂ (40 vol% 45 vol%) and non-methane organic compounds (NMOCs < 1%, containing sulfur compounds, halogenated hydrocarbons, aromatic hydrocarbons, aliphatic hydrocarbons, terpenes and oxidizing compounds, etc.). CH₄ is considered to be the second largest greenhouse gas in the world, its warming potential is 27.9 times that of CO₂, and its contribution to Greenhouse Effect of the world is more than 30%^[1,2]. In addition, as the main source of odor in landfills, NMOCs has many kinds of pollutants, complex components and strong toxicity, and many components have "three-cause" effect and genetic toxicity, which pollute the overlying soil and its surrounding environment for a long time, seriously endangering human health and ecological safety^[3]. Therefore, the effective control and removal of disordered landfill gas discharge from landfills has always been a hot spot in the field of solid waste.

Under landfill gas acclimation, a variety of functional microorganisms are derived from the overburden soil, which plays an important role in landfill gas removal. At the same time, the establishment of cover vegetation can enhance the degradation of landfill gas by functional microorganisms, which has important application potential in effectively controlling landfill gas emissions. The study on the synergistic degradation of methane by vegetation and microorganisms in landfill cover has been carried out. Xiaoli Chai et al. [4] investigated the types of vegetation in closed landfills and their effects on methane emission reduction, and found that the methane concentration in the covered soil near the rhizosphere of *Phragmites australis* was significantly lower than that in bare soil. Thomas G et al. [5] found that the methane oxidation effect under the combined action of *Alfalfa* and *Grass* was the best, which was significantly higher than that of *White poplar* and *Mango grass*. Bian Rongxing et al. [6] found that the CH₄ emission from the vegetation cover area in the landfill is only 1/2 of that in the bare area. Stralis-Pavese et al. [7] found that the CH₄ oxidation capacity of bare soil was less than 18 g/(m² · d), while that of CH₄ was higher than 35 g/(m² · d) under *Alfalfa* and *Grass* cover. These studies fully proved that vegetation has a significant effect on methane oxidation capacity of landfill cover soil, and vegetation roots and rhizosphere microorganisms play an important role in controlling methane emission.

At present, almost all studies have only carried out root microbial enhanced CH₄ degradation for one or several limited vegetation, and no effective vegetation selection list has been obtained. Under the influence of climatic environment and tolerance, many problems have not been solved, such as the complex growth of vegetation in the natural state of landfill soil, which vegetation has stronger adaptability in landfill, and which vegetation has better control effect of landfill gas pollution.

Artificial establishment of landfill cover vegetation has become an important means of landfill gas control in closed landfills, and the optimization of vegetation is an important prerequisite for artificial planting. During the operation of the landfill site, the natural growth vegetation was constantly stressed by landfill gas, and the pollutant tolerance and degradation ability of continuously domesticated plants [8-12]. The application of natural vegetation in landfill closure has important potential for methane emission reduction. However, there are few

studies focusing on the synergistic action of vegetation and microorganisms to control methane emissions, and there are few reports on vegetation species information, rhizosphere microecological characteristics, bio-oxidation capacity and metabonomic characteristics during landfill operation.

Based on this, this study systematically investigated the vegetation of unclosed landfills under the subtropical monsoon humid climate in Chongqing, China, and sampled and analyzed the roots and rhizosphere soil of different vegetation. The root characteristics, rhizosphere microecological characteristics, methane oxidizing bacteria community composition, microbial community distribution and root depth of different vegetation were investigated, and based on the methane oxidation ability of rhizosphere soil, the effects of different vegetation on the utilization of microbial methane in the rhizosphere were investigated, and the metabolites composition and distribution of typical vegetation rhizosphere soil were investigated by metabonomics technology. Finally, the relationship between vegetation type, rhizosphere microecological characteristics and biological oxidation capacity was established systematically, and the primary vegetation with high methane degradation efficiency was selected, which is expected to provide theoretical guidance for plant establishment in situ restoration project to control methane emission.

2 MATERIALS AND METHODS

2.1 Landfill investigation and root soil sampling

The municipal solid waste landfill in Rongchang District of Chongqing is located in Qibaoyan Village, Yuan Town, Rongchang District, with an effective landfill capacity of 19.847 × 10⁶m³, a daily treatment capacity of 260t and a service life of 15 years. The structure diagram is shown in Figure 1. The landfill is located in the subtropical monsoon climate region, with an annual average temperature of 7~12 °C and a relative humidity of 87%. Vegetation investigation and soil sampling are carried out in the cover layer of the landfill for more than 2 years to avoid the contingency of the survey results.

The vegetation cover characteristics of the landfill were investigated, and the plants with good

growth were selected for plant sampling and rhizosphere soil collection. Under the condition of ensuring the integrity of the plant root as much as possible, dig the plant, remove a large piece of soil from the root, and measure the root length of different vegetation. The rhizosphere soil was

carefully extracted, placed on ice and transported to the laboratory for immediate treatment. The soil samples were stored in -80 °C refrigerator for DNA extraction and soil physical and chemical properties detection.



Fig. 1 schematic diagram of municipal solid waste landfill site in Rongchang District, Chongqing

2.2 Determination of methane oxidation capacity of vegetation rhizosphere soil

15 g soil samples of vegetation roots were put into a series of 100 mL clean serum bottles, sealed with aluminum lid and lined with polytetrafluoroethylene silica gel gasket, and the air in the bottle was replaced by 20 mL CH₄ (about 2080 ppmv). All samples were in triplicate. The serum bottle system was cultured in a 30 °C biochemical incubator, and the change of methane concentration was determined continuously.

2.3 Rhizosphere soil microbial DNA extraction and diversity sequencing

Soil samples of root system of cover vegetation were stored in the environment of -80 °C for microbial diversity analysis. The total genomic DNA of microorganisms was extracted by Mobio PowerSoil® DNA Isolation Kit, and the DNA sample was purified by Mobio PowerClean® DNA Clean-Up Kit. The purified DNA products were detected by 1% agarose gel electrophoresis.

The 16s DNA hypervariable region sequence was sequenced, and the sequencing region was V3+V4. Use Trimmomatic and FLASH software to process the Miseq sequencing data to obtain clean data: (1) Filter the

bases with a quality value of less than 20 in the read tail, set the window of 50 bp, if the average value in the window is less than 20 bp, truncate the back base from the window, and filter the read; below 50 bp after quality control. (2) According to the overlap relationship between PE reads, the paired reads will be merge into a sequence, and the minimum overlap length is 10 bp. (3) The maximum mismatch ratio of the overlap region of the splicing sequence is 0.2, and the screening does not conform to the sequence; (4) Detect the box sequence at the end of the sequence, the minimum mismatch number is 0, reverse complement the sequence including box at the beginning, and remove the barcode from the box; (5) Detect the sequence and distinguish the sample, the barcode mismatch number is 0, and the maximum primer mismatch number is 2.

The data error and correlation were analyzed by SPSS Statistics 21 software, and the principal component analysis and diversity data were analyzed by i-sanger platform (<http://www.i-sanger.com/>).

2.4 Sequencing of microbial metabonomics in rhizosphere soil

The soil samples of different vegetation roots were measured and analyzed by LC-MS non-targeted metabonomics, and the original data were imported into metabonomics processing software Progenesis QI (Waters Corporation,

Milford, USA). At the same time, the MS information was matched with metabolic common database HMDB (<http://www.hmdb.ca/>) and Metlin (<https://metlin.scripps.edu/>) database.

The preprocessed data is uploaded to Meiji Biological Cloud platform (<https://cloud.majorbio.com>) for data analysis. The R software package ropls (Version1.6.2) carries out principal component analysis (PCA) and orthogonal least square discriminant analysis (OPLS-DA), and uses 7 cycles of interactive verification to evaluate the stability of the model. In addition, student's t test and multiple of difference analysis were performed. The selection of significant differential metabolites was based on the variable weight value (VIP) obtained by OPLS-DA model and the p value of student's t test. The metabolites with $VIP > 1$ and $p < 0.05$ were significant differential metabolites. By constructing the volcano map of differential metabolites, the number of metabolites was significantly up-regulated, down-regulated and no significant change.

Then, the metabolic pathways of all the differential metabolites were analyzed, and the KEGG (<https://www.kegg.jp/kegg/pathway.html>) metabolic pathways of the differential metabolites were constructed. At the same time, the metabolic pathways involved in the metabolites were visually analyzed by iPath3.0 (<http://pathways.embl.de>) to view the metabolic pathway information of the whole biological system.

Sample preparation method: take 50 mg solid sample or 100 μ L liquid sample into 1.5 mL centrifuge tube, add 400 μ L extract (acetonitrile: methanol = 1:1), after vortex mixing for 30 s, extract 30 min (5 $^{\circ}$ C, 40 KHz) by low temperature ultrasonic extraction, place the sample in -20 $^{\circ}$ C, 30 min, 4 $^{\circ}$ C, 13000 g centrifuge 15 min, remove the supernatant, dry with nitrogen, and redissolve in 120 μ L resolution (acetonitrile: water = 1:1). The samples were extracted by low temperature ultrasonic extraction with 5 min (5 $^{\circ}$ C, 40 KHz), 4 $^{\circ}$ C and 13000 g centrifugation for 5 min. The supernatant was removed to the injection vial with intubation and analyzed on the machine.

The LC-MS analysis instrument platform is Semefeld's ultra high performance liquid chromatography tandem Fourier transform mass spectrometry UHPLC-Q Exactive HF-X system.

The chromatographic conditions were as

follows: ACQUrTY UPLC HSS T3 (100mm \times 2.1mm I.D.) , 1.8 μ m; Waters, Milford, USA); mobile phase A is 95% water + 5% acetonitrile (containing 0.1% formic acid), mobile phase B is 47.5% acetonitrile + 47.5% isopropanol + 5% water (containing 0.1% formic acid), the injection volume is 2 μ L, and the column temperature is 40 $^{\circ}$ C.

3 RESULTS AND DISCUSSION

3.1 Vegetation distribution and community characteristics of landfills during landfill

In the process of landfill, the environment of the landfill is bad, the garbage is constantly landfill and compacted, the garbage is placed in the open before the soil and film is covered, and the landfill gas produced by natural fermentation continues to spread to the surrounding area. The vegetation growth environment of the whole landfill is complex and bad, so that the vegetation coverage of the cover is low and the plant grows slowly naturally. The vegetation cover during the operation of Chongqing Rongchang Municipal solid waste Landfill site in western China was investigated. Samples were taken in winter and summer every year, and the growth of vegetation was observed for three consecutive years. Almost no vegetation grows in all the garbage dumps, and most of the vegetation grows in the soil cover around the garbage dumps. The vegetation coverage is higher in summer, the vegetation height is lower, the growth cycle is longer, the winter coverage is lower, the plant height is higher, and the life cycle is shorter. In addition, the composition of typical vegetation communities is investigated, and the results are shown in Figure 2. The air and soil where the natural vegetation is located are stressed by malodorous landfill gas and landfill leachate for a long time, and the natural vegetation community evolves to herbaceous vegetation that is easy to survive, and there are few trees and shrubs. A total of 22 species were found, of which herbaceous vegetation such as *Goosegrass Herb*, *Cynodon Dactylon*, *Zizania Latifolia*, *Mugwort*, *Miscanthus Floridulus*, *Wild Chrysanthemum* and *Cyperus Glomeratus L.* are growing well.

The physical and chemical properties of rhizosphere soils with different vegetation show that the pH value of rhizosphere soil of each vegetation is between 6.16 and 7.42, and the pH value of rhizosphere soil of most vegetation is lower than that of non-rhizosphere soil; the water content of rhizosphere soil of each vegetation is between 9.24% and 35.82%, and the moisture content of non-rhizosphere soil is 23.69%, which

will significantly affect the water content of rhizosphere soil under the action of vegetation. The secretory components of rhizosphere are related to plant species and growth stage, in which acid ion and hydrogen ion can change the pH value of soil rhizosphere environment. Soil under acidic condition is more conducive to organic matter desorption and promote the absorption of pollutants by plant roots^[13]. The effect of soil moisture content on the degradation of pollutants

in rhizosphere soil is more complex. Soil water can inhibit the adsorption of pollutants on the surface of soil particles and promote bioavailability. However, too much water will inhibit plant nutrient transport in the rhizosphere. Affect plant growth and degradation ability^[14,15]. Thus it can be seen that vegetation varieties and root characteristics are important factors to change the physical and chemical properties of rhizosphere soil.

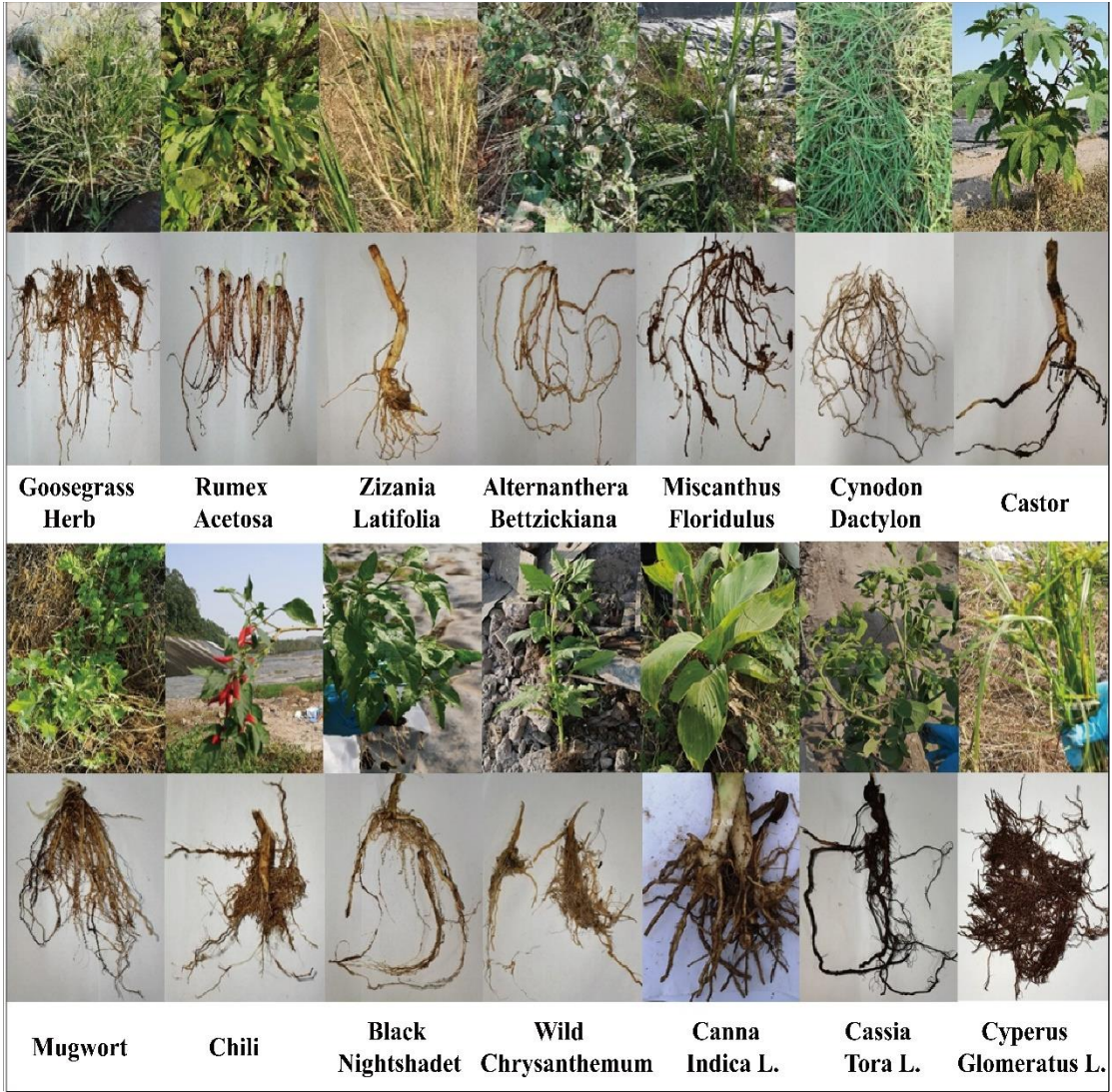


Fig. 2 Different vegetation and root structure of landfill cover

3.2 Analysis of methane oxidation ability of rhizosphere soil under different vegetation

Under the influence of rhizosphere exudates, the microbial activity of rhizosphere soil of some vegetation increased. Based on the ability of methane oxidation, the microbial activities in the rhizosphere of different vegetation were investigated, and the results are shown in Figure

3. Overall, the average methane degradation ability of rhizosphere soil of each vegetation was higher than that of non-rhizosphere soil. The methane oxidation efficiency of rhizosphere soil of *Reed*, *Amaranthus Spinosus*, *Cassia Tora L.* and *Black Nightshadet* decreased gradually after 48 hours, while that of other vegetation increased continuously. The methane oxidation efficiency of *Rumex Acetosa* rhizosphere soil was the highest at 72 h, which was 90.32%. The rate of

methane oxidation in the rhizosphere soil of *Reed* and *Goosegrass Herb* was the lowest, which was $0.0133 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$ and $0.0113 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$. These vegetation were not suitable for enhancing methane oxidation in landfills, while those of *Rice*, *Black Nightshadet*, *Broussonetia Papyrifera* and *Cassia Tora L.* rhizosphere soil were further enhanced, which were $0.0556 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$, $0.0459 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$, $0.0305 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$ and $0.0355 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$. The methane oxidation rate of *Rumex Acetosa* rhizosphere soil was the strongest, which was $0.0800 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$, which was nearly 20 times higher than that of non-rhizosphere soil.

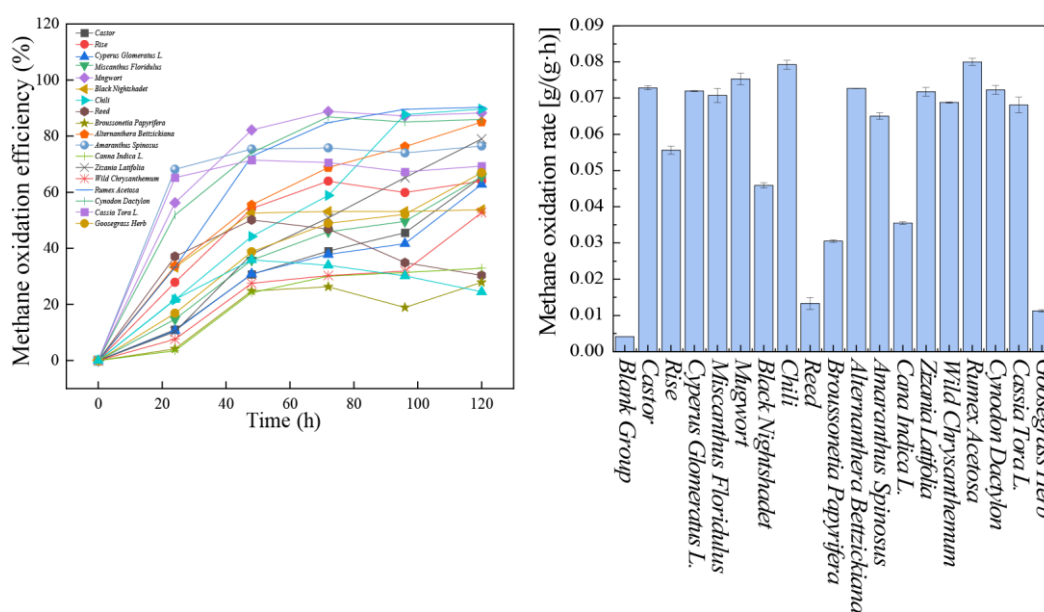


Fig. 3 methane bio-oxidation ability of rhizosphere soil of different vegetation.
A: Methane biooxidation efficiency; B: Methane biodegradation rate.

3.3 Analysis of microbial diversity in the rhizosphere of different vegetation

3.3.1 Analysis of microbial diversity in the rhizosphere of vegetation: The diversity of soil microorganisms in the rhizosphere of typical vegetation was sequenced. A total of 1130239 sequences were obtained from all samples, and 49431 to 64394 sequences were obtained from a single sample (Average = 56511.95). Except for *Reed*, *Cynodon Dactylon*, *Goosegrass Herb* and *Black Nightshadet*, the microbial abundance in the rhizosphere soil of other vegetation was higher than that in non-rhizosphere soil, and the Chao index of *Zizania Latifolia* and *Miscanthus Floridulus* rhizosphere soil was the highest, 3964.40 and 3897.03, respectively. In addition, the Shannon index of rhizosphere microorganisms of each plant was higher than that of non-rhizosphere soil (Shannon=4.45), and

According to the methane oxidation rate, the promoting effect of vegetation on methane oxidation in rhizosphere soil can be divided into three grades: "slight promotion" ($0.0000-0.0200 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$), "general promotion" ($0.0200-0.0600 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$) and "strong promotion" ($0.0600-0.1000 \text{ g}_{\text{CH}_4}/(\text{kg}_{\text{soil}} \cdot \text{h})$). The above results show that plant root exudates can promote methane biological oxidation to a certain extent, and the methane oxidation ability of rhizosphere soil of different vegetation is quite different. "strong promotion" vegetation such as *Rumex Acetosa*, *Mugwort* and *Chili* is beneficial to enhance methane oxidation in landfills.

Miscanthus Floridulus had the largest Shannon index (Shannon=6.83). Thus it can be seen that the landfill cover soil under the action of vegetation can enrich a variety of microorganisms and effectively improve the uniformity and diversity of microbial community. Some studies have shown that vegetation root exudates and oxygen transport in the rhizosphere can change the physical and chemical properties of soil, provide a suitable growth environment for some functional microorganisms, enhance the activity of functional microorganisms, and induce microbial enrichment in plant rhizosphere microdomains^[16,17].

3.3.2 Analysis of microbial Community composition in Rhizosphere soil of different vegetation: Bray-Curtis clustering and microbial community at the rhizosphere soil gate level showed that the microbial community structure of

Black Nightshadet and *Goosegrass Herb* rhizosphere soil was the most similar to that of non-rhizosphere soil. *Wild Chrysanthemum*, *Canaa Indica L.*, *Castor*, *Rumex Acetosa*, *Mugwort*, *Amaranthus Spinosus*, *Cyoeus Glomeratus L.*, *Chili*, *Cassia Tora L.*, *Zizania Latifolia*, *Rise*, *Miscanthus Floridulus*, *Broussonetia Papyrifera* and *Alternanthera Bettzickiana* plant rhizosphere microbial community cluster distance is closer, *Cynodon Dactylon* and *Reed* have the same cluster tree. Under the action of vegetation, the microbial community structure of the covered soil was quite different, and the dominant bacteria were Proteobacteria, Acidobacteriota, Chloroflexi, Actinobacteria, Acidobacteriota, Gemmatimonadota and Bacteroidota. The maximum relative abundance of Proteobacteria appeared in the non-rhizosphere soil (50.79%), the maximum relative abundance of Acidobacteriota in the rhizosphere soil of *Cynodon Dactylon* (32.76%), the maximum relative abundance of Chloroflexi in the rhizosphere soil of *Reed* (18.99%), the maximum relative abundance of Actinobacteria in the *Castor* rhizosphere soil (16.02%), and the maximum relative abundance of Acidobacteriota in the *Cynodon Dactylon* rhizosphere soil (32.76%). The maximum relative abundance of Gemmatimonadota and Bacteroidota in the rhizosphere soil of *Reed* and *Black Nightshadet* were 18.49% and 22.57% respectively. The relative abundance of Acidobacteriota, Chloroflexi and Actinobacteriota can be increased under the action of vegetation.

The structure of horizontal microbial community is shown in Figure 4A. The dominant bacteria in non-rhizosphere soil was *Methylocaldum*, accounting for 28.94%. Under the action of vegetation, the dominant bacteria in each rhizosphere soil changed one after another, and the evenness increased significantly. The dominant bacteria in the rhizosphere soil of *Cynodon Dactylon* were *norank_f_norank_o_Vicinamibacterales*, *YC-ZSS-LKJ147* and *norank_f_norank_o_Subgroup_7*; and the dominant bacteria in *Mugwort* rhizosphere soil were *Sphingomonas*, *unclassified_o_Rhizobiales* and *Steroidobacter*. The dominant bacteria in *Castor* rhizosphere soil were *unclassified_f_Methylomonadaceae*, *norank_f_Gemmatimonadaceae* and *Sphingomonas*; The dominant bacteria in *Rumex Acetosa* rhizosphere soil were *Methylocaldum*, *unclassified_f_Methylomonadaceae* and *Methylocaldum*. Many studies have found that *Methylocaldum*, *unclassified_f_Methylomonadaceae*,

Methylocaldum and other strains are the key microorganisms in the process of methane bio-oxidation in soil, and play an important role in the transformation of pollutants. In addition, there are some interaction effects between the dominant bacteria such as *norank_f_norank_o_Vicinamibacterales* and *Dongia* and the corresponding vegetation, which indirectly promote the methane use efficiency of rhizosphere soil, but the specific interaction mechanism needs to be further explored.

3.3.3 Analysis of enrichment effect of methane oxidizing bacteria community:

Methane oxidizing bacteria is considered to be one of the most critical functional strains in the mulch layer. The characteristic methane oxidizing bacteria community in the rhizosphere of vegetation was analyzed, as shown in Figure 4B. The total abundance of methane oxidizing bacteria community in non-rhizosphere soil was significantly higher than that in rhizosphere soil samples. *Methylophilaceae* as the dominant methane oxidizing bacteria in non-rhizosphere soil, its relative abundance was as high as 28.94%. After the induction of *Rumex Acetosa* vegetation, the dominant methane oxidizing bacteria in rhizosphere soil was transformed into *Methylocaldum*, and the relative abundance reached 6.27%. According to the methane bio-oxidation ability of the rhizosphere soil of each vegetation, it was inferred that some vegetation induced the dominant methane oxidizing bacteria to colonize in the rhizosphere microdomain, and enhanced the methane bio-oxidation ability of the rhizosphere soil under the effect of vegetation-microbial interaction. *Rumex Acetosa*, *Castor*, *Mugwort* rhizosphere soil can significantly enrich *unclassified_f_Methylomonadaceae*, *Methylocaldum*; *Rice*, *Miscanthus Floridulus* rhizosphere soil can effectively enrich *Methylobacter*; *Goosegrass Herb* rhizosphere soil can significantly enrich *Methylobacillus*; *Amaranthus Spinosus*, *Broussonetia Papyrifera* can effectively enrich *Methylocystis*; *Black Nightshadet* can significantly enrich *Methylococcus*. Thus it can be seen that the inducing effect of vegetation on rhizosphere microorganisms is specific, and the difference in the composition of plant root exudates may be the main reason for inducing the colonization of methane oxidizing bacteria in the rhizosphere. Previous studies have shown that *Methylocaldum* can efficiently assimilate methane through the Embden-Meyerhof-Parnas pathway [18]. The comprehensive genome-scale metabolic model of Villada J C et al [19] has proved that *Methylocaldum* has a variety of methane utilization strategies. Therefore, *Methylophilaceae* is not the main function of

structure with vegetation root depth: The root length of vegetation affects the process of gas mass transfer in the covered soil, which in turn affects the distribution of functional strains. Based on the root characteristics of *Rumex Acetosa* and *Mugwort*, the microbial community structure at different root depths was investigated, and the results are shown in Figure 3D. The results showed that the average length of *Rumex Acetosa* root system was 15.4cm. The relative abundance of Proteobacteria and Chloroflexi was the highest in 15 cm *Rumex Acetosa* root system, in which the relative abundance of Proteobacteria was as high as 22.6%. The structure of microbial community in the rhizosphere of *Mugwort* root was significantly different between 5cm and 10cm, and the dominant phylum changed from Proteobacteria, Acidobacteria, Chloroflexi to Proteobacteria, Bacteroidota, Acidobacteria. In addition, Firmicutes was only detected in 15 cm and 20 cm *Mugwort* rhizosphere soil and 20 cm

Rumex Acetosa Rhizosphere soil. Combined with the investigation of root length, it was speculated that root oxygen transport increased oxygen concentration in rhizosphere microdomain, and the biological activity of anaerobes Firmicutes was inhibited and the abundance was low. Many scholars have also done some research on Firmicutes, Christian et al ^[20] isolation of Firmicutes from anaerobic biomass hydrolysis; Subirats J et al ^[21] has been proved that Firmicutes is more suitable for anaerobic environment.

In addition, there were significant differences in the community structure of methane oxidizing bacteria under different root depths between *Rumex Acetosa* and *Mugwort*. *Methylobacterium* can be enriched in the rhizosphere microdomain of 5-10 cm *Mugwort* and 5-15cm *Rumex Acetosa*, while the enrichment effect of methane oxidizing bacteria is not strong in other root depths. It is speculated that *Methylobacterium* is the main functional bacteria that can enhance the ability of methane oxidation in rhizosphere soil [22,23]. Combined with the physical and chemical properties of rhizosphere soil, *Rumex Acetosa* root exudates promote the acidity of rhizosphere soil, and the enrichment of methane oxidizing bacteria is beneficial to the removal of organic pollutants.

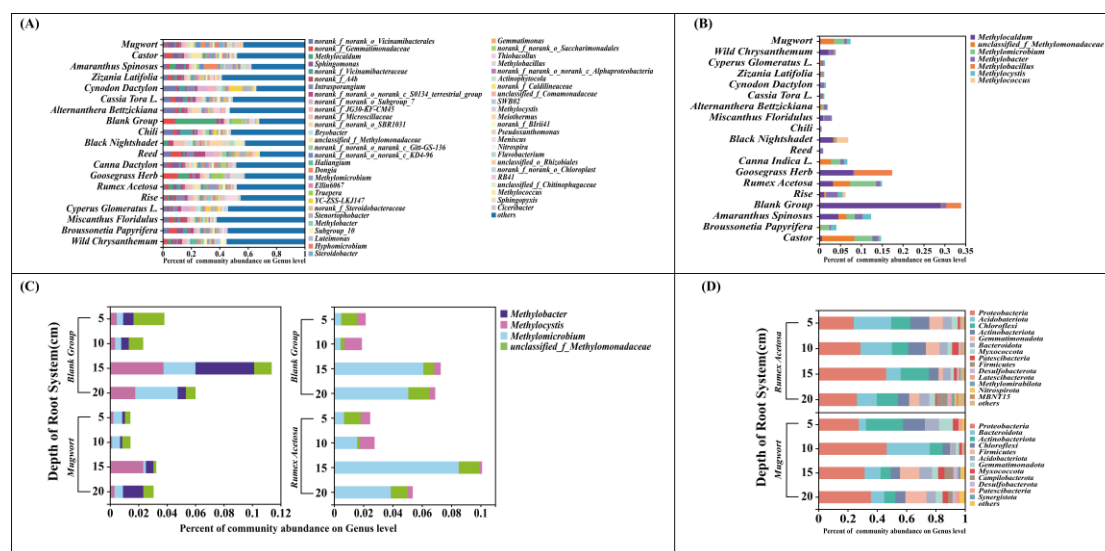


Fig. 4 Microbial community structure in the rhizosphere of natural vegetation in landfill cover.
A: Relative abundance of rhizosphere microorganisms of each vegetation; B: Relative abundance of methane oxidizing bacteria in rhizosphere microorganisms of each vegetation; C: Distribution of methane oxidizing bacteria under different root depths of *Rumex Acetosa* and *Mugwort* vegetation; D: Gate-level microbial diversity under different root depths of *Rumex Acetosa* and *Mugwort*.

3.4 Metabolomics analysis of microorganisms in the rhizosphere of vegetation

3.4.1 Analysis of microbial metabonomics

data: *Rumex Acetosa*, *Amaranthus Spinosus*, *Broussonetia Papyrifera* and *Goosegrass Herb* rhizosphere soil were selected as typical samples to carry out LC-MC non-targeted metabonomic analysis to explore the metabolic map differences among the samples. A total of 6253 positive ion mode peaks and 5579 negative ion mode peaks were selected to complete the follow-up screening. The quality control (QC) samples were clustered in the center, indicating the good reproducibility and stability of the equipment in the whole study. In order to determine the possible discrete points, the overall distribution trend of all samples was studied by PCA analysis (Figure 5A). All groups showed changes in positive and negative ion patterns respectively, and there were significant differences among the four groups of samples. This shows that the type of vegetation is an important reason for the difference of metabolites in different rhizosphere soils. The verification results of PLS-DA model show that there is no over-fitting phenomenon in the model. The number of microbial metabolites in the rhizosphere of four kinds of vegetation was obtained by comparative Venn map analysis. A total of 499 rhizosphere microbial metabolites were obtained from the rhizosphere soil samples of four kinds of vegetation, of which the total number of metabolites was as high as 388. The specific rhizosphere microbial metabolites of *Rumex Acetosa*, *Amaranthus Spinosus*, *Broussonetia Papyrifera* and *Goosegrass Herb* were 14, 7, 19 and 3 respectively.

3.4.2 Qualitative and quantitative analysis of microbial metabolism in rhizosphere:

According to HMDB 4.0 database (<http://www.hmdb.ca/>), 596 metabolites were identified in ESI+ and ESI- mode. The chemically classified number of certain HMDB metabolites accumulated at the highest frequency in each rhizosphere microbiome. 596 metabolites are chemically classified into 239 lipids and lipid molecules, 83 organic acids and derivatives, 71 organic oxides, 71 organic heterocyclic compounds, 64 phenylpropionic acids and polyketones, 28 benzene compounds, 22 nucleosides, nucleotides and analogues, 6 organic nitrogen compounds, 5 alkaloids and derivatives, 3 hydrocarbons, 2 lignans, neolignans and related compounds. One organic sulfide and 1 metal / non-metal mixed compound. The clustering of metabolites in the sample is shown in figure 4F. The main metabolites enriched in *Rumex Acetosa* rhizosphere soil are Fluvoxamino acid, 1-

Formylneogrifolin, 3-[3,4-dihydroxy-2-(8-hydroxy-3,7-dimethylocta-2,6-dien-1-yl)phenyl]propanoic acid, Limonexic acid, Lucidenic acid A, Trigoformin. The main metabolites enriched in the rhizosphere soil of *Broussonetia Papyrifera* were 2-Hydroxycinnamic acid, 6-[(2-carboxyacetyl)oxy]-3, 4,5-trihydroxyoxane-2-carboxylic acid, Cinnamic acid; the clustering of metabolites in the rhizosphere soil of *Broussonetia Papyrifera* and *Amaranthus Spinosus* was not significant. Yang L ^[24] used Cinnamic acid as autotoxin to investigate the effect of Cinnamic acid on microbial community structure in the rhizosphere of vegetation under salt stress. The results showed that Cinnamic acid aggravated the effect of salt stress at high concentration, but reduced it at low concentration. Cinnamic acid concentration of 50 mg·kg⁻¹ could effectively alleviate the effect of salt stress on bacterial community. Therefore, the microorganisms in the rhizosphere of *Broussonetia Papyrifera* resist salt stress in the overlying soil by secreting Cinnamic acid and its derivatives, but their inhibitory effect on the biological process of methane oxidizing bacteria ^[25], reduces the abundance of methane oxidizing bacteria in the rhizosphere soil of *Broussonetia Papyrifera*. As a result, the ability of methane oxidation in the rhizosphere soil is low. In contrast, *Rumex Acetosa* rhizosphere microorganisms promote their own TCA cycle through Limonexic acid in root exudates and promote the metabolic efficiency of microorganisms in the rhizosphere environment. Microbial metabolites can further induce the biosynthesis of plant secondary metabolites and plant hormones, which is beneficial to vegetation synthesis of nicotine and other alkaloids, while promoting the carbon sequestration process of prokaryotes ^[28]. So as to improve the methane oxidation ability of rhizosphere soil ^[29].

Compared with the microbial metabolites in the rhizosphere of *Goosegrass Herb* with low methane oxidation ability, the specific metabolites of *Rumex Acetosa*, *Amaranthus Spinosus* and *Broussonetia Papyrifera* rhizosphere microbial communities are mainly lipids, organic acids and their derivatives, organic oxides, phenylpropionic acids and polyketones, while there are little differences in metabolites such as metal / non-metallic compounds, organic nitrogen compounds, alkaloids and derivatives. A total of 327 differentially expressed proteins were identified in the rhizosphere soil of four kinds of vegetation ($P < 0.05$, $Vip > 1$, as shown in Figure 4B-Figure 4D). It was found that there were three substances with the most significant differences in metabolites, which were 11 α ,17 β -Dihydroxy-1,4-androstadien-3-one,

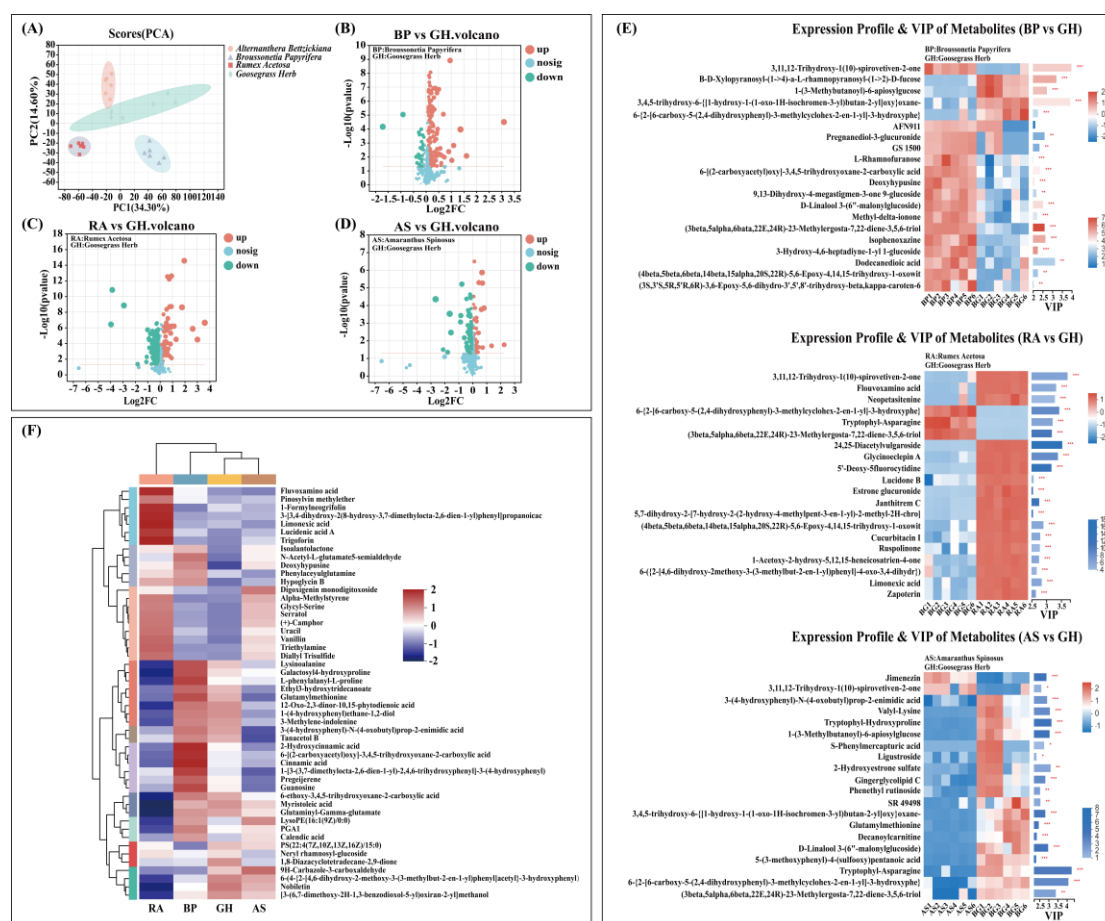


Fig. 5 Soil metabolomics analysis of typical vegetation rhizosphere in landfill.

A: PCA score map of typical vegetation rhizosphere soil samples ($P < 0.05$), B-D: volcanic difference map of typical vegetation rhizosphere soil metabolism, E: relative abundance of microbial metabolism in typical vegetation rhizosphere (significant level defined as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$), F: comparison of relative abundance of different metabolites between typical vegetation rhizosphere microbial community and control group.

The differential metabolites of rhizosphere microorganisms of *Rumex Acetosa*, *Amaranthus Spinosus* and *Broussonetia Papyrifera* vegetation were analyzed, as shown in Figure 4E. Compared with *Goosegrass Herb*, except Asparagine A, Tryptophan-asparagine, Valine-lysine and Neopeptide xetine, all metabolites were more in the rhizosphere microbial samples of *Rumex Acetosa* vegetation. Among them, 3,11,12-Trihydroxy-1 (10)-spirovetive-2-one, 24,25-Diacetylulgaroside and Glycinoeclepin A significantly contributed to the differential metabolism between *Rumex Acetosa* rhizosphere microorganisms and *Goosegrass Herb* Rhizosphere microorganisms. The metabolism of rhizosphere microorganisms in *Amaranthus Spinosus* and *Broussonetia Papyrifera* was significantly different from that in the rhizosphere, but the abundance of specific metabolites was not

high. Thus it can be seen that the root exudates of *Rumex Acetosa* induce the production of specific metabolites of rhizosphere microorganisms, resulting in changes in the community structure and metabolic characteristics of rhizosphere microorganisms, resulting in higher methane oxidation efficiency in *Rumex Acetosa* rhizosphere soil.

3.4.3 Metabolic Pathway Analysis based on KEGG Database: The results of metabolite classification are shown in Figure 6D and Figure 6E. A total of 14 kinds of KEGG Compound were detected in soil samples of vegetation roots, and the corresponding statistical maps of Pathway pathways were divided into 7 categories, in which Metabolism Processes accounted for the largest proportion and Cellular Processes accounted for

the smallest. It is inferred that the vegetation root system can effectively enhance the metabolic intensity of microorganisms in the rhizosphere and accelerate the utilization efficiency of substances in the rhizosphere microdomain. Microbial metabolites are an important medium of allelopathic effect with plants, and can feedback regulate plant growth state [30-32].

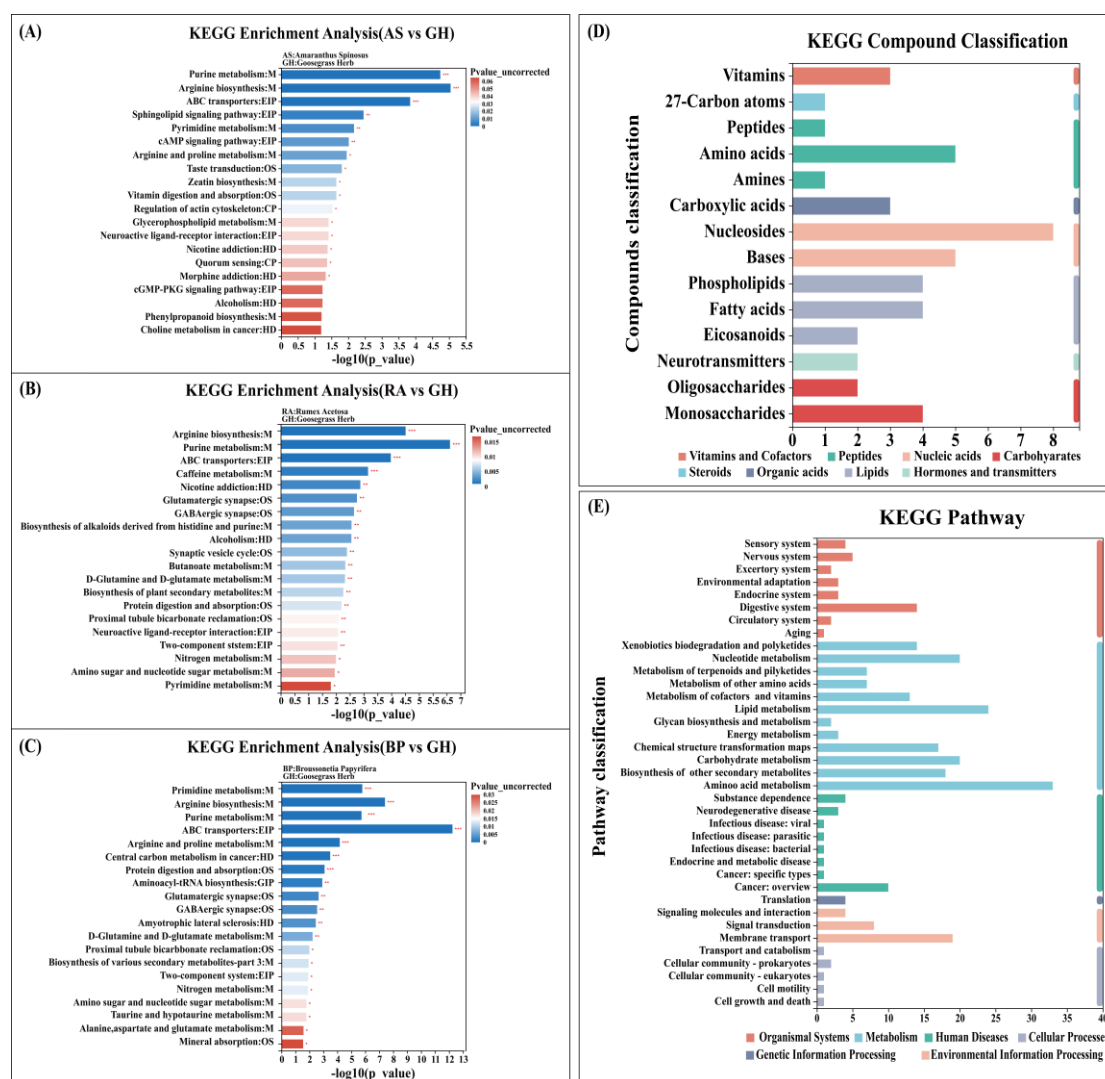


Fig. 5 Analysis of microbial metabolic pathway in the rhizosphere of typical vegetation.

A: 20 KEGG enrichment path item ratios obtained from *Rumex Acetosa*, *Amaranthus Spinosa* and *Broussonetia Papyrifera* rhizosphere microorganisms and Rhizosphere microorganisms; D: Rhizosphere soil samples taxonomic columnar statistical chart (Note: Vertical coordinate is KEGG compound classification, Abscissa is annotated to the number of compounds of this kind; Bar color indicates that it belongs to the first class of compounds) E: Statistical map of Pathway in rhizosphere soil samples (Note: the ordinate is the secondary classification of KEGG metabolic pathway, and the Abscissa is the number of compounds annotated to this pathway. KEGG metabolic pathways can be divided into seven categories: Metabolism, Genetic Information Processing, Environmental Information Processing, Cellular Processes, Organismal Systems, Human Diseases, and Drug Development. The color of the bar indicates different metabolic pathways.)

The most obvious and important metabolic or biosynthetic pathways related to metabolism were identified according to the KEGG database. The KEGG database identified 67 metabolic pathways and annotated 42 very important metabolic pathways. The differential metabolites of four vegetation rhizosphere microorganisms are involved in the following metabolic or biosynthetic pathways: the biosynthesis of Arginine, the metabolism of Taurine and Sub-aurine, the metabolism of Alanine, Aspartic acid and Glutamic acid, and the metabolism of Arginine and Proline.

The detailed relationship between different metabolic pathways and microorganisms was studied by comparing the results of microbial KEGG pathway enrichment in the rhizosphere of *Goosegrass Herb*. Figure 6 A-C shows 20 path items with the highest KEGG enrichment in the rhizosphere microbial communities of three kinds of vegetation. The characteristics of differential metabolites were observed in the following metabolic or biosynthetic pathways in the comparison of microbial metabolic pathways in the rhizosphere of *Amaranthus Spinosus*, *Rumex Acetosa* and *Goosegrass Herb*: Arginine biosynthesis: M, Purine metabolism: M and ABC transporters: EIP. In the comparison of microbial metabolic pathways in the rhizosphere of *Broussonetia Papyrifera* and *Goosegrass Herb* group, the characteristics of differential metabolites were observed in the following metabolic or biosynthetic pathways: Pyrimidine metabolism: M, Arginine biosynthesis: M, Purine metabolism: M, ABC transporters: EIP, Arginine and proline metabolism: M, Central carbon metabolism in cancer: HD and Protein digestion and absorption: OS ($P < 0.01$). It was also found that the metabolic or biosynthetic pathways with the highest enrichment rate in each control group were the Regulation of actin cytoskeleton, Nicotin addiction and Arginine biosynthesis. When Kateryna et al. [33] studied the effect of root exudates on microbial activity, it was found that rhizosphere microorganisms preferred to eat aromatic organic acids secreted by plants Nicotinic, Shikimic, Cinnamic acid and Indole-3-

acetic. Thus it can be seen that the nicotine contained in the root exudates of *Rumex Acetosa* promotes the metabolism or biosynthesis pathway of rhizosphere microorganisms to be enriched in the process of Nicotine, which improves the metabolic ability of rhizosphere microorganisms and obtains higher methane oxidation efficiency and this phenomenon also exists in the rhizosphere microorganisms of amaranth.

4 CONCLUSION

The rhizosphere soil of natural vegetation in landfill has a specific inducing effect on methane oxidizing bacteria, which can effectively improve the biological oxidation capacity of methane in landfill gas, and systematically establish the relationship among vegetation type, rhizosphere microecological characteristics and biological oxidation capacity. *Rumex Acetosa* changed the physical and chemical properties and oxygen content of rhizosphere soil through root exudates and root oxygen transport, which could significantly induce the colonization of methane oxidizing bacteria *Methylobacterium* in rhizosphere microdomain, and the methane oxidation efficiency of *Rumex Acetosa* rhizosphere soil was as high as 90.32%. *Rumex Acetosa* promotes the TCA cycle of rhizosphere microorganisms through Limonexic acid in root exudates, strengthens the metabolic efficiency of rhizosphere microorganisms and the process of biological carbon sequestration, and its metabolites further induce the biosynthesis of plant secondary metabolites and plant hormones. Root exudates regulate the community structure and metabolic characteristics of rhizosphere microorganisms by inducing specific metabolic processes of rhizosphere microorganisms, so as to obtain higher methane bio-oxidation ability in rhizosphere soil. Therefore, the extensive planting of *Rumex Acetosa* and other vegetation in the landfill cover is of great significance to control the disorderly emission of landfill gas.

REFERENCES:

- [1] Change Intergovernmental Panel on Climate. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change[M]. Cambridge: Cambridge University Press, 2021
- [2] Hao L M, Zhong J S, Zhang H H, et al. The release and influencing factors of ch₄ in solid waste landfill spring and summer[J]. Environmental Sciences, 2009, 22(1): 83-88.
- [3] Li C Y, Zhang B C. Greenhouse gas of influencing global climate and research current situation of greenhouse calibration gas in our country[J]. Metrology and Measurement Technique, 2005, 32(4): 34-35.
- [4] Xiaoli C, Xin Z, Ziyang L, et al. Characteristics of vegetation and its relationship with landfill gas in closed landfill[J]. Biomass and bioenergy, 2011, 35(3): 1295-1301.
- [5] Reichenauer T G, Watzinger A, Riesing J, et al. Impact of different plants on the gas profile of a landfill cover[J]. Waste Management, 2011, 31(5): 843-853.
- [6] Bian R, Xin D, Chai X. Methane emissions from landfill: influence of vegetation and weather conditions[J]. Environmental technology, 2019, 40(16): 2173-2181.
- [7] Stralis-Pavese N, Sessitsch A, Weilharter A, et al. Optimization of diagnostic microarray for application in analysing landfill methanotroph communities under different plant covers[J]. Environmental Microbiology, 2004, 6(4): 347-363.
- [8] Zhang X Y, Tian Z Y, Zhang C, et al. Research progress on rhizosphere effect mechanism of phytoremediation of polycyclic aromatic hydrocarbons contaminated soil [J]. Soil Bulletin, 2021 (052,005).
- [9] RANĐELOVIĆ D, GAJIĆ G, MUTIĆ J, et al. Ecological potential of *Epilobium dodonaei* Vill. for restoration of metalliferous mine wastes[J]. Ecological Engineering, 2016, 95: 800-810.
- [10] Zu Y Q, Lu X, Zhan F D, Hu W Y, Li Y. Research progress on the role and mechanism of arbuscular mycorrhizal fungi in phytoremediation of soil contaminated by heavy metals [J]. Acta Physiologica Sinica, 2015 51 (10): 1538-1548.
- [11] Shi Y F, Wang S M, Guo J N, et al. Effects of arbuscular mycorrhizal inoculation on the phytoremediation of PAH-contaminated soil: A meta-analysis[J]. Chemosphere, 2022(03), 136033,
- [12] Cheng Y, Wang J H. Study on functional difference of bacterial community in plant rhizosphere in contaminated soil [J]. Environmental Science and Technology, 2022J 45 (05): 84-91.
- [13] Miao X Y, Zhou Q X. Research progress on factors affecting phytoremediation efficiency of contaminated soil [J]. Journal of Ecology, 2015, 34 (03): 870-877.
- [14] Yen N C, Daniel P. S, Root exudates impact plant performance under abiotic stress[J], Trends in Plant Science, 2022, 27(01), 80-91.
- [15] Charlotte V, Manhattan L, Nicolas H, et al. How does soil water status influence the fate of soil organic matter? A review of processes across scales[J], Earth-Science Reviews, 2022, 104214.
- [16] Elohim B-B, Damar L-A, Thelma Y., et al. Conquering compacted soils: uncovering the molecular components of root soil penetration[J], Trends in Plant Science, 2022, 27(08), 814-827.
- [17] Yang F, Huang M B, Li C H, et al. Vegetation restoration increases the diversity of bacterial communities in deep soils[J], Applied Soil Ecology, 2022, 180,104631.
- [18] Villada J C, Duran M F, Lim C K, et al. Integrative genome-scale metabolic modeling reveals versatile metabolic strategies for methane utilization in *Methylobacterium album* BG8. Cold Spring Harbor Laboratory, 2021.
- [19] Fu Y, Yi L, Lidstrom M. The oxidative TCA cycle operates during methanotrophic growth of the Type I methanotroph *Methylobacterium buryatense* 5GB1.[J]. Metabolic Engineering, 2017, 42:43-51.
- [20] Christian, Abendroth, Sarah, et al. Complete Genome Sequence of a New Firmicutes Species Isolated from Anaerobic Biomass Hydrolysis[J]. Genome Announcements, 2017, 5(40).
- [21] Subirats J, Sharpe H, Topp E. Fate of Clostridia and other spore-forming Firmicute bacteria during feedstock anaerobic digestion and aerobic composting[J]. Journal of Environmental Management, 2022, 309:114643-.
- [22] Li L, Li Y C, Liu D P, et al. Research progress on biodegradation of refractory organic compounds based on methane oxidizing bacteria [J]. Environmental Chemistry, 2020 (2): 8.
- [23] Zhang H, Xing Z L, Wang J, et al. Research status, microbial metabolic characteristics and prospect of heterotrophic assimilation degradation of chlorinated hydrocarbons [J]. Journal of Biological Engineering, 2020, 36 (6): 18.
- [24] Yang L. Effects of Cinnamic Acid on Bacterial Community Diversity in Rhizosphere Soil of Cucumber Seedlings

- Under Salt Stress[J]. Agricultural Sciences in China, 2010.
- [25] Sun H J, Wang Q. Effect of Benzoic Acid and Cinnamic Acid on Watermelon Seeding Root Cell Protective Enzymes and Membrane Permeability[J]. Acta Agriculturae Boreali-Occidentalis Sinica, 2007.\
- [26] Liang Z T, Tang T. Effects of endophytes on biosynthesis and stress resistance of plant secondary metabolites [J]. Biotechnology Bulletin, 2021, 37 (8): 11.
- [27] Lv Z Y, Sun W J, Jiang R, et al. Phytohormones Jasmonic Acid, Salicylic Acid, Gibberellins, and Absciscic Acid are Key Mediators of Plant Secondary Metabolites[J]. World Journal of traditional Chinese Medicine, 2021, 7(3):19.
- [28] Christopher J, Alteri, Stephanie D , et al. Anaerobic respiration using a complete oxidative TCA cycle drives multicellular swarming in *Proteus mirabilis*. [J]. mBio, 2012.
- [29] Ming-Hua Y, Hong-Gen D, Jiang Y, et al. GC/MS Metabonomics Analysis of *Dioscorea bulbifera* L. Microtubers Conserved in vitro at Low Temperature[J]. Bulletin of Botanical Research, 2018.
- [30] Lin Z M, Muhammad U K, Fang C X, et al. Types of crop allelopathy: research status and prospects in China[J]. Chinese Journal of Ecological Agriculture, 2022.
- [31] Troedsson U. Signalling between plants and microorganisms[J]. Cell & Organism Biology Lund University Sölvegatanb Lund Sweden, 2005.
- [32] Tian J H, Rao S, Gao Y, et al. Wheat straw biochar amendment suppresses tomato bacterial wilt caused by *Ralstonia solanacearum*: Potential effects of rhizosphere organic acids and amino acids[J]. Journal of Integrative Agriculture, 2021, 20(9):2450-2462.
- [33] Zhalnina K, Louie K B, Hao Z, et al. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly[J]. Nature microbiology, 2018, 3(4): 470-480.