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Research on the impact of exogenous pollutants on the soil environment and their remediation mechanisms and models

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Keywords: Tall fescue; cadmium pollution; phytoremediation; low molecular organic acids

Abstract: In recent years, with the rapid development of industrialisation, soil heavy metal pollution is increasing, of which cadmium pollution is particularly prominent. Cadmium pollution not only leads to crop root damage, photosynthesis inhibition, but also indirectly affects the safety of human life, remediation of cadmium contaminated soil is urgent. As one of the phytoremediation materials, tall fescue is effective in remediating cadmium-contaminated soil.In this study, we used the cadmium-resistant tall fescue variety "Airey 3" as the material. It was subjected to various concentrations of cadmium stress, and within the same cadmium concentration, different concentrations of citric acid, oxalic acid, and acetic acid were applied. We conducted a comprehensive investigation into the impact of cadmium pollution on the soil environment, aiming to explore the remediation capacity and mechanism of tall fescue in cadmium-contaminated soil. Additionally, we analyzed the relationship between low-molecular-weight organic acids and the root-associated microbiota.The correlation between low molecular organic acids and inter-root microbial community structure, soil enzyme activity and other indicators was analysed. The results showed that all the physical and chemical indicators of the soil were under different degrees of stress under the adversity, and the total microbial amount decreased by 60.12%, the community structure changed, and the enzyme activity, soil nutrient and organic matter content decreased significantly. And certain low molecular organic acid can effectively enhance the repair ability of tall fescue, the maximum average increase in soil organic matter was 119.12%~142.55%, effective phosphorus and quick-acting potassium content increased by 32.87% and 34.58%. In summary, tall fescue has a certain role in the remediation of cadmium-contaminated soil, and the application of exogenous organic acids can effectively enhance the soil remediation capacity of tall fescue in cadmium-stressed environments, which provides a theoretical basis for promoting the management of cadmium pollution in soils and improving the value of cadmium-contaminated soil development and utilisation.

1. Introduction

With the rapid advancement of industrialisation and urbanisation, the irrational use of chemical

fertilisers and pesticides, and sewage irrigation, heavy metal pollution of farmland soils has intensified. According to the National Pollution Survey Bulletin, 19.4% of farmland soil in China is polluted by heavy metals, of which cadmium (Cd) is the most important pollutant [19]. Cadmium is a toxic heavy metal and is characterised by high mobility and transport[28], which can cause osteoporosis, cancer and kidney dysfunction[11]. Phytoremediation is considered an emerging environmentally friendly technology to remove heavy metals from the environment[7]and has become a hotspot in modern soil remediation research. Tall fescue (*Festuca elata* Keng ex E. B. Alexeev), a perennial tufted herb in the grass family Fescue, has been shown to be cadmium-tolerant and possesses a good enrichment capacity. However, during the restoration process, high-intensity stress effects can cause tall fescue to suffer from growth slowdown and biomass reduction, leading to a decrease in restoration efficiency[25]. Therefore, there is an urgent need for in-depth investigation of methods to mitigate heavy metal toxicity in tall fescue, in order to promote the adaptation of tall fescue to adversity and enhance the efficiency of cadmium enrichment, so as to better contribute to the battle of pollution prevention and contro[1].

Low molecular *weight organic* acids *(*LMWOAs) can chelate with heavy metals to form complexes to alleviate inter-root toxic effects and also participate in the uptake of heavy metals [26]. Niu Xueli[15] found that the addition of LMWOAs effectively reduced the effects of cadmium stress and improved the germination rate of tall fescue seeds by studying the root secretion of vetiver grass. However, in the current research on cadmium enrichment in tall fescue, there is no report on the physiological and biochemical effects of LMWOAs on tall fescue under cadmium stress and their regulatory mechanisms. Therefore, in this project, we selected tall fescue as the experimental material for remediation of cadmium-contaminated soil, screened out the cadmium concentration of tall fescue with the best remediation effect, and sprayed it with different concentrations of low molecular organic acids (LMOAs) under the corresponding intensity of cadmium stress, and monitored the changes of soil indexes, in order to find out the influence of LMWOAs on the remediation ability of tall fescue under cadmium stress, and to provide a reference for the improvement of the cadmium-enrichment effect of tall fescue.

2. Materials and methods

2.1 Experimental materials

A series of pre-tests were conducted to determine the Cd-tolerant cultivar of tall fescue (Airey 3), the Cd concentration gradient (0 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg, 300 mg/kg), the type of low molecular organic acids (citric acid CA, oxalic acid OA, acetic acid AA) and the concentration gradient (10 mmol/kg, 20 mmol/kg) of low molecular organic acids and the concentration gradient of low molecular organic acids (10 mmol/kg, 20 mmol/kg). In the experiment to investigate the remediation concentration of cadmium, five experimental groups were set up with 14 d as the remediation cycle. The soil indicators before pollution, before remediation, and after remediation were tested for comparison. In the experiment of exploring the low molecular organic acids to promote the restoration efficiency of tall fescue, under the condition of 100 mg/kg Cd pollution, the three low molecular organic acids of the two concentrations mentioned above were added respectively, and seven experimental groups were set up, in which the CK group used deionised water as the control, and three parallel control groups were set up in each. After 14d of treatment, the roots of tall fescue were also taken to contact the soil for testing and analysis, the results are shown in Table 1.

Table 1: Concentrations of exogenous organic acids applied under 100 mg/kg Cd stress

deal with	Cadmium content (mg/kg)	Types of organic acids	Acid concentration (mmol/kg)
CK	100	/	/
CA_{10}	100	citrate	10
CA_{20}	100	citrate	20
OA_{10}	100	oxalic acid $C_2H_2O_4$	10
OA_{20}	100	oxalic acid $C_2H_2O_4$	20
AA_{10}	100	acetic acid (CH_3COOH)	10
AA_{20}	100	acetic acid (CH_3COOH)	20

2.2 Experimental methodology

The potentiometric method [1] was used to determine soil pH. The hydrated thermal potassium dichromate oxidation-colorimetry method [22] was employed to determine soil organic matter content. The dilution-plate method [5] was utilized to detect soil microbial species and numbers. Potassium permanganate titration [18] was conducted to determine catalase content. The sodium phenol-sodium hypochlorite colorimetry method was applied to measure urease content. The 3,5-dinitrosalicylic acid colorimetry method was used to determine sucrase content. The alkaline hydrolysis distillation method was adopted to ascertain soil hydrolytic nitrogen content. The sodium bicarbonate method was employed to determine soil available phosphorus. The sodium tetraphenylboronate turbidimetry method [14] was used to measure soil quick-acting potassium. Atomic absorption spectrophotometry [6] was utilized to determine soil cadmium metal content.

2.3 Data processing

The experimental data were collated using Microsoft Excel 2019, and significant differences were analysed by calculating the mean and standard error by one-way ANOVA (one-way ANOVA) and least significant difference (LSD) method using SPSS 25.0, and the significant differences were analysed using Origin 2022 software for graphing.

3. Results and analyses

3.1 Effect of tall fescue restoration, organic acids-tall fescue on soil physico-chemical indicators

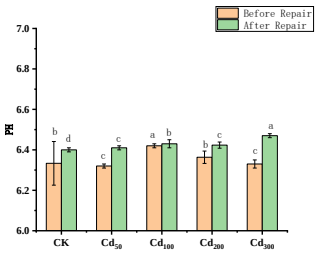

Figure 1: Comparison of soil pH data before and after remediation

Soil pH is an important property of soil and an important factor affecting soil fertility. Soil pH not only has a non-negligible impact on the biological reactions and life activities of microorganisms, but also has a close relationship with the growth and production of crops. As can be seen from the figure 1, figure 2 and figure 3, cadmium pollution has little effect on soil pH. With the increase of acetic

acid, oxalic acid and citric acid, the soil pH decreased by about 0.2~0.3 units, in which the degree of influence contrasted: citric acid > oxalic acid > acetic acid. And pH increased after restoration by tall fescue.

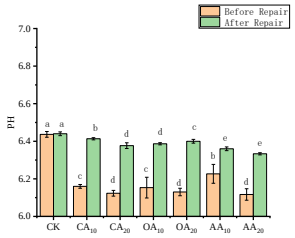

Figure 2: Comparison of pH data before and after application of organic acids

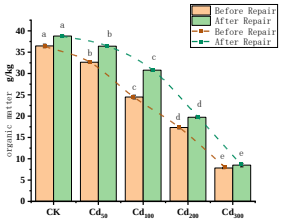

Figure 3: Comparison of organic matter before and after restoration

The content of soil organic matter showed a trend of first slow decrease and then rapid decrease with the increase of Cd concentration. After the restoration of tall fescue, the organic matter increased by 5.59%, 12.29%, 24.76%, 11.45%, and 7.78%, respectively, with the most significant increase in the Cd_{100} group. LMWOAs increased the soil organic matter content, and the same concentration of oxalic acid increased the organic matter content by about 119.12%-142.55% compared with the other low molecular organic acids, whereas the increasing effect of citric acid and acetic acid had no significant difference. In conclusion, cadmium stress greatly reduced the organic matter content, while the collaboration of tall fescue and organic acids significantly alleviated the cadmium stress.

3.2 Effects of tall fescue restoration, organic acids-tall fescue on soil microorganisms

The number of soil microorganisms directly affects the biochemical activity of soil and the composition and transformation of soil nutrients, which is one of the important indicators of soil fertility. As can be seen from Figure 4 to Figure 7, the numbers of bacteria, fungi and actinomycetes decreased significantly under cadmium stress. And with the increase of cadmium concentration, the decrease was more obvious, among which the bacteria had the largest decrease of 24.69%~63.76%. Therefore, it can be obtained that the degree of tolerance of soil microorganisms to cadmium is shown as fungi>actinomycetes>bacteria, and metallic cadmium has a significant inhibitory effect on the growth of microorganisms.

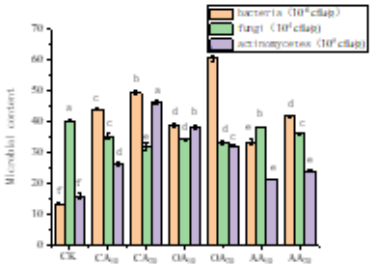

Figure 4: Microbial changes before and after organic acid application

Compared with CK, the cultivation of tall fescue all increased the number of microorganisms, and the increases of bacteria, fungi and actinomycetes were 20.27%, 23.45% and 16.96%. With the increase of Cd concentration, the promotion effect of tall fescue on microorganisms showed a tendency of increasing and then decreasing, and reached the maximum value at Cd_{100}.

The effects of different LMWOAs on soil microorganisms varied significantly, with an increase in both bacterial and actinomycetes at low concentrations of acid, with the most significant effect of citric acid treatment, which reached a maximum increase of 322.85% in the CA_{10} group, and a decrease in fungal content. The promotional effect of acid was further enhanced at high concentrations, with oxalic acid having a relatively prominent effect. It can be concluded that tall fescue and organic acids are able to detoxify the toxic effects of cadmium through the increase of microorganisms.

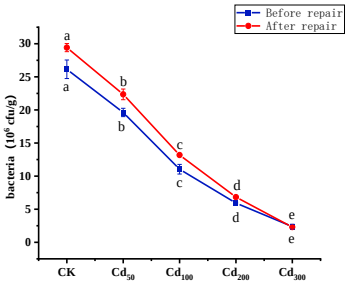

Figure 5: Comparison of bacteria before and after restoration of tall

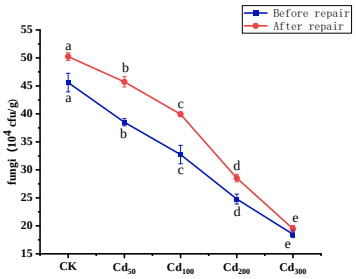

Figure 6: Comparison of fungi before and after tall fescue restoration

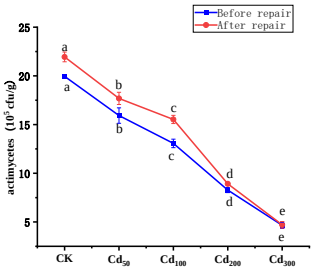

Figure 7: Comparison of actinomycetes before and after tall fescue restoration

3.3 Effects of tall fescue restoration, organic acids-tall fescue on soil nutrients

Hydrolysable nitrogen (HN) and quick-acting potassium (AK) in the soil will inhibit the crop's absorption of Cd, while effective phosphorus (AP) plays a promotional role [29], and the level of soil nutrient indexes directly responds to the degree of soil Cd stress. As shown in Table 2, soil HN content increased significantly under Cd treatment, reaching a maximum value of 75.01% in Cd_{50}, and AP

and AK content decreased; after restoration of tall fescue, HN content decreased in all groups, reaching 58.35% in Cd_{100}, and AP and AK content increased by 32.87% and 34.58%, respectively. 3 kinds of low molecular organic acids had no significant correlation with soil HN, and positively correlated with AP. In Cd_{100} and AA_{20}, the maximum increase was 52.50%. In conclusion, Cd stress concentration and soil available potassium (AK) and available phosphorus (AP) content are negatively correlated, while humic acid (HN) content is positively correlated. Tall fescue has a good repair and improvement effect on cadmium-contaminated soil, and its ability to do so is largely influenced by the species and concentration of exogenous organic acids.

Table 2: Comparison of nitrogen, phosphorus and potassium (NPK) data before and after restoration of tall fescue and comparison of NPK of different organic acid species

	HN		AK		AP	
	pre-restoration	after repair	pre-restoration	after repair	pre-restoration	after repair
CK	61.97±2.13d	55.84±1.25e	283.84±2.16a	277.51±1.64a	94.51±1.65a	89.94±2.48a
Cd_{50}	80.44±1.98c	62.78±1.54e	168.22±1.79b	190.64±1.65b	49.81±2.44b	60.38±2.46b
Cd_{100}	140.77±1.85b	99.41±3.24d	108.94±2.13c	169.56±1.88d	34.65±2.56d	52.65±2.14d
Cd_{200}	170.86±1.64a	114.57±2.46b	73.54±2.11d	120.51±1.42e	29.38±2.46d	42.54±2.21e
Cd_{300}	164.51±2.46a	87.49±1.94e	62.65±2.46d	78.84±2.43f	31.5±2.95d	44.11±2.46e
CK	140.77±1.87b	99.41±1.63d	108.94±2.15c	169.56±2.32d	34.65±2.42d	52.65±2.88d
CA_{10}	137.84±2.14b	105.48±1.56c	111.55±2.46c	170.51±2.64d	36.75±2.46c	54.87±1.58c
CA_{20}	144.51±1.64b	127.75±2.15a	114.75±1.98c	171.23±2.25d	37.21±2.55c	54.99±1.59c
OA_{10}	139.48±2.75b	98.71±2.55d	109.89±1.46c	171.21±2.48d	40.35±1.49c	59.71±1.85b
OA_{20}	138.77±2.49b	103.84±1.46c	110.18±1.02c	172.51±2.66d	52.84±1.56b	62.31±2.90b
AA_{10}	144.85±2.45b	94.49±2.46d	112.32±1.87c	175.98±1.84d	38.59±2.42c	56.87±2.95c
AA_{20}	142.18±2.46b	104.38±1.45c	115.84±2.95c	181.35±1.56c	37.84±2.48c	54.42±2.16c

3.4 Effect of tall fescue and organic acids on enzyme activity

The size of soil enzyme activity value comprehensively reflects the soil physicochemical properties and heavy metal concentration, which has important monitoring value for reflecting soil heavy metal pollution [12]. Figures 8~Figure 13 show that after the soil was contaminated by Cd, the activities of catalase and sucrase decreased, but not as obvious as urease, which reached a maximum decrease of 26.13% in Cd_{50} . The activities of various enzymes in the soil increased significantly after the restoration of tall fescue, and the activities of all enzymes could reach the maximum after the treatment of LMWOAs, and the promotion of enzymes was the most prominent by CA, which promoted the urease to reach a significant level, and the activity increased by 78.95% compared with that of CK. The activity was 78.95% higher than that of CK. In summary, Cd pollution will lead to the reduction of soil enzyme activity, the most significant effect on urease, tall fescue remediation can effectively enhance the activity of various enzymes to alleviate cadmium stress, and organic acids can enhance the ability of tall fescue remediation of cadmium-contaminated soils by promoting the recovery of enzyme activity.

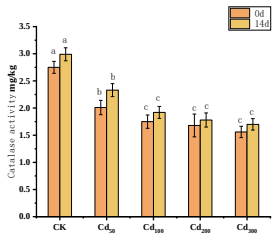

Figure 8: Comparison of catalase before and after tall fescue restoration

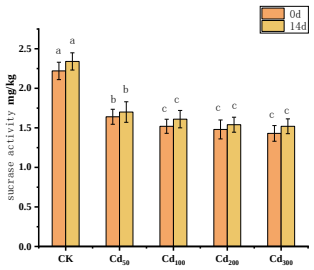

Figure 9: Comparison of urease before and after tall fescue restoration

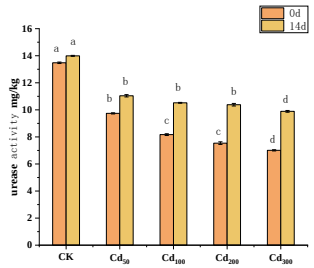

Figure 10: Comparison of sucrase before and after restoration of tall fescue

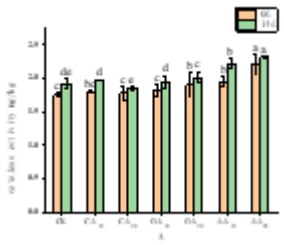

Figure 11: Comparison of catalase before and after the application of organic acids

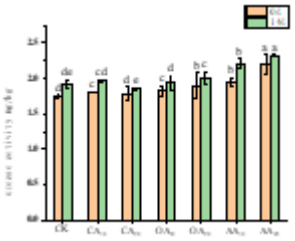

Figure 12: Comparison of urease before and after organic acid application

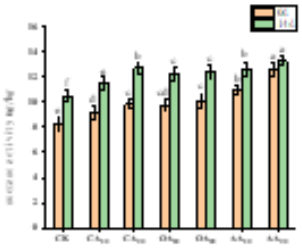

Figure 13: Comparison of sucrase before and after the application of organic acids

3.5 Effect of Tall Fescue Restoration, Organic Acid-Tall Fescue on Metal Content

Plant roots are able to secrete LMWOAs in the inter-root soil, thus enhancing the mobility as well as the bioavailability of the metal, and increasing the uptake and enrichment of heavy metals by the plant as a whole[4]. As can be seen from *Figure.* 14, the Cd concentration in the soil decreased significantly after the treatment of tall fescue, and reached the maximum reduction at Cd_{100} , indicating that tall fescue has an uptake effect on a certain concentration of Cd, and that the treatment was most effective at about 100 mg/kg. As can be seen from Figure 15, a certain concentration of organic acids can promote the uptake of heavy metals in the subsoil of tall fescue, with 20 mmol/kg oxalic acid treatment having the strongest effect, while 20 mmol/kg acetic acid had the weakest effect. This indicates that the application of LMWOAs significantly increased the cadmium accumulation capacity of tall fescue and the removal efficiency of soil cadmium.

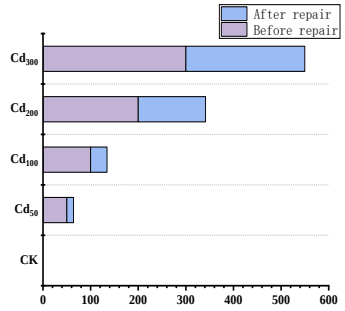

Figure 14: Before and after comparison of soil Cd content before and after tall fescue remediation

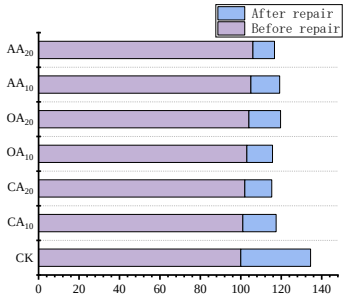

Figure 15: Comparison of soil cadmium content before and after organic acid application.

4. Discussion

Soil physicochemical properties are important indicators of soil health, and pH is a key indicator of soil acidity and alkalinity, which plays an important role in soil regulation and plant growth [8]. NO^{3-} -N uptake by tall fescue was dominant in this experiment, leading to a reduction in the proportion of ammonium nitrogen, which helps to increase soil pH and alleviate soil acidification. The data showed that the addition of LMWOAs significantly reduced soil pH, and it can be hypothesised that due to the decrease in pH, the exchange of heavy metal cations adsorbed on the surface of the soil solid phase with H^+ subsequently increased, and heavy metal ions in the soil solid phase were transferred to the soil liquid phase [20], and the imposition of LMWOAs in the present experiments increased inter-root uptake of cadmium in the absorbable form, and facilitated the uptake and accumulation of Cd in the plant body, which is consistent with the experimental results of Yang Shao'e [27].

Tall fescue increased soil organic matter to alleviate Cd stress.Chen[3] found that organic matter can directly interact with Cd^{2+} through hydrogen bonding, ligand exchange and complexation to

increase the content of soil effective state Cd. In this experiment, the soil organic matter content of all experimental groups was improved and the content of effective state Cd increased[21]. And the application of exogenous acid had a promoting effect on Cd activation and morphological transformation, which led to the transformation of residual state Cd to acid-soluble state Cd[2], which was then adsorbed by plant root cells[10], thus reducing the Cd capacity.

The inhibition of microorganisms by metal ionic substances begins with the toxic inhibition of cell-related enzymes[13]. The inhibition of one active enzyme may lead to the paralysis of the whole chain of degradation or metabolic reactions, which in turn leads to the inhibition of the overall activity of the cell, and may ultimately lead to cell death[16]. Cadmium concentration was positively correlated with the inhibitory effect on microorganisms in this experiment, and the inhibitory effect varied greatly among different species due to the different corresponding enzyme contents of different bacteria or the different corresponding physiological processes they were involved in. In this experiment, tall fescue enhanced Cd uptake by improving the inter-root soil environment and adjusting the number and structure of microorganisms[9]. Low molecular organic acids (LMWOAs) can provide sufficient carbon, nitrogen and other nutrients for the growth and reproduction of inter-root microorganism. It was explored that all the three LMWOAs were found to promote microorganisms, and the acid concentration was positively correlated with the number of microorganisms in the set concentration range, which is in line with the findings of Qiuying Wang[17].

Soil enzymes are the core of soil ecosystems and are involved in important ecological processes such as organic matter decomposition and soil microbial energy acquisition. In this study, it was found that the activities of three soil enzymes showed a decreasing trend with the increase of Cd concentration, so it is reasonable to assume that Cd changed the spatial structure or primary structure of enzymes and made them inactive[30]. Planting of tall fescue significantly increased catalase, sucrase and urease activities, improved soil nutrient content and promoted decomposition of organic matter. Organic acids in this study enhanced soil enzyme activities by increasing soil organic matter, supplying energy to microorganisms and thus enhancing soil enzyme activities, which is inconsistent with the conclusion that citric acid promotes urease activity in black soil while oxalic acid and acetic acid have inhibitory effects on the enzyme activities as concluded by Pengzhi Zhao, which may be due to the nature of the soil and the presence of special components. The analysis showed that the hydrolysable nitrogen content increased under Cd stress, while the fast-acting potassium and effective phosphorus content decreased. This is inconsistent with the conclusion of Wu Sijia[24] that cadmium stress increased the total nitrogen, effective phosphorus and fast-acting potassium content of two varieties of rice soils, which may be due to the fact that cadmium may be toxic to soil microorganisms, reducing the rate of nitrogen decomposition leading to the accumulation of nitrogen, and at the same time, chelating of Cd with phosphorus to form insoluble compounds, which reduces phosphorus effectiveness.

5. Summary and outlook

In summary, the soil physicochemical indexes changed under cadmium adversity, and its microbial number and enzyme activity decreased, tall fescue has a certain adsorption effect on cadmium in the soil, and the application of exogenous low molecular organic acids can enhance the root system of tall fescue's uptake capacity and transport capacity of heavy metal cadmium under cadmium stress environment, so as to comprehensively strengthen the remediation effect of tall fescue on the soil. This experiment preliminarily confirmed that tall fescue could reduce the toxicity by accumulating most of the absorbed heavy metals in the roots or transporting them to the aboveground part through the absorption of roots. The root system of tall fescue secretes special organic substances, such as a certain organic acid to promote the dissolution of heavy metal elements in the soil and root uptake.

Therefore, the application of exogenous LMWOAs can effectively improve the soil remediation effect of tall fescue under cadmium stress, thus increasing the microbial population, organic matter content and enzyme activity and other indicators.

With the rapid development of China's economy, the problem of soil cadmium pollution brought about by industrial production and other aspects has been intensifying, which has a drastic impact on the agricultural economy, and tall fescue, as an important turfgrass with strong resistance, has great potential for development in the field of soil remediation[23]. Our team has experimentally demonstrated that tall fescue can reduce the degree of cadmium pollution in soil through root uptake, and that LMWOAs can effectively enhance the effect of tall fescue in alleviating cadmium toxicity. As a result, a multi-dimensional comprehensive evaluation system was initially established to explore the effects of cadmium stress on tall fescue, the ability of tall fescue to adsorb cadmium and the optimal concentration of LMWOAs applied. The synthesis concluded that the effect of tall fescue combined with exogenous LMWOAs on soil remediation was superior to the single use of tall fescue, and then utilised its own absorption and removal of heavy metals and other pollutants in the soil. This provides a practical basis for solving the cadmium pollution problem and improving the restoration rate of arable land, effectively promotes the comprehensive development of ecological and economic benefits and comprehensive value of tall fescue, promotes the green and sustainable development of tall fescue-related industries and soil remediation, and deeply implements the concept of "pollution reduction and emission reduction, green development".

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