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EFFECTS OF MIXING ADDITION OF THE GREENERY WASTES ON THEIR REMEDIATING EFFECTS ON PETROLEUM POLLUTED SOIL

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Abstract: In this study, greenery wastes of Platycladus orientalis (Po) and the other 5 species which are commonly used in urban landscaping were collected. Monospecific greenery wastes and their 1:1 mixtures containing Po waste were used to treat contaminated soil with a petroleum content of 15.00 g/kg, to conduct a 150-day laboratory remediating experiment at 20-25℃, under a soil moisture of 50% of the saturated water holding capacity. The effects of mixed addition of greenery wastes on their remediating effects on contaminated soil were detected, to provide scientific basis for the reasonable utilization of greenery waste in contaminated soil remediation. The results indicated that: (1) Mixed addition of Po waste with Picea asperata (Pa), Acer truncatum (At), Ginkgo biloba (Gb) or Juniperus formosana (Jf) waste significantly enhanced their effect of removing petroleum contaminants, especially for removing aromatic and non-hydrocarbon substances; while the mixed addition of Po waste with Pinus tabuliformis (Pt) waste significantly weakened their remediating effects. The mixed addition of Po waste with Gb or Jf waste significantly enhanced their effects of replenishing soil available N and P; the mixed addition of it with Pa, Pt, Gb or Jf waste tended to enhance their effects of stimulating the activities of soil invertase, urease, phosphatase and dehydrogenase, while the mixed of it with At waste tended to weaken their simulating effects. (2) Mixed litters with high contents of C, N, terpenoids and amino acids, and chemical dispersion facilitated the degradation of petroleum contaminants, while those with high contents of soluble saccharides and total organic acids/terpenoids led to contrast effects; mixed litters with high N content facilitated the increment of soil enzymatic activities, while those with high contents of total organic acids and terpenoids led to contrast effects. In conclusion, mixed addition of Po and Jf, Po and Gb, and Po and Pa wastes might enhance their overall remediating effects on petroleum contaminated soil, while mixed addition of Po and Pt wastes might weaken their ability of removing the contaminants, and this mixing form of greenery wastes should be avoided.

Key words: greenery waste; mixed decomposition; non-additive effects; degradation rate of contaminants; soil biological and chemical properties

1. INTRODUCTION

In recent years, with the rapidly increased demand for petroleum resources in transportation, industry, agriculture and other fields, frequent leakage accidents in the process of crude oil production, transportation and refining have caused continuous adverse impacts on the soil environment in relevant areas (Ergozhin et al., 2020). This also promoted the development of a variety of petroleum-contaminated soil remediation technologies. Among them, bioremediation methods, such as phytoremediation and microbial bioremediation, have gradually attracted the attention of researchers due to their characteristics of less secondary contamination and low cost. However, these technologies are also limited by the site environment, the tolerance and biomass of remeditors, and the competition between them and indigenous organisms (Hui & Wang, 2018; Zheng et al., 2021). Necrophytoremediation is one of the emerging techniques developed in recent years, which uses plant residues to provide key nutrients (such as N and P) and auxiliary substances (such as phenolics, soluble saccharides, terpenoids, amino acids, flavones and organic acids, etc.) to indigenous soil remediation microbes, accelerating their growth and the cometabolic degradation of contaminants, and increasing the bioavailability of contaminants to them

(Koshlaf et al., 2016a; Koshlaf et al., 2020; Shahsavari et al., 2015). It also indirectly introduces some potential remediating microbes (Koshlaf et al., 2020), and thus promotes the degradation of contaminants through both biostimulation and bioenhancement pathways, and avoids the limiting factors faced by the aforementioned technologies. Existing studies have confirmed that the treatments on contaminated soil using various plant residues, such as non-economic biomass of crops (such as straw, corn cob and peel, Koshlaf et al., 2020; Llado et al., 2015; Wu et al., 2011), plant litters (Zhang et al., 2020) and urban greenery wastes (Han et al., 2017) significantly promote the degradation of petroleum or its aromatic hydrocarbon products, and restoring the damaged chemical and biological properties of soil to a certain extent. Of course, how to improve the remediation effects of plant residues further remains to be studied.

According to its mechanism, the efficiency of necrophytoremediation might be limited by the following factors. First, due to the differences in the ecophysiological characteristics of the source plants, plant residues exhibit different chemical composition. The specialization of the types and contents of nutrients and auxiliary substances (e.g., the lack of N or P) makes it difficult for a monospecific plant residue to support the growth of various contaminants-degrading microbes at same time and improve their degradation efficiency. Second, some plant residues containing amounts of degradation auxiliary substances (such as phenolic substances) decompose quite slow, thus they can hardly stimulate the degradation of contaminants before they are adsorbed by soil organic matters (Hou et al., 2021; Qin et al., 2018), so that their potential in remediation cannot be fully played. Many studies have shown that when plant residues are mixed, the differences in chemical properties and the improvement of microenvironment might significantly accelerate the decomposition of each other, accelerating the release of nutrients and secondary metabolites (Kou et al., 2020b; Qin et al., 2018), and thus significantly improving soil microbial biomass and diversity (Chen et al., 2020; Li et al., 2020). Therefore, it might be feasible to improve the remediation efficiency of contaminated soil by simply treating it with mixed plant residues, and correspondingly, reduce the consumption of plant residue resources. However, existing studies on necrophytoremediation mainly focus on the detection of the effects of monospecific residues in stimulating soil contaminants degradation, and their remediating effect of key soil biological and chemical indicators, while the attention on the remediation effect of mixed plant residues addition are still rare. Hence, the validity of the abovementioned hypothesis still needs further research.

Urban greenery waste is one of the potential biomass resources that are widely produced along with the continuous expansion of urbanization and the developoment of urban green infrastructures (Yu et al., 2015; Vasilescu et al., 2022). It has huge biomass, but the collection of it only leads to quite limited interference to the natural environment. Compared with other plant residues, greenery waste is derived from both plant pruning and natural shedding of plant organs in the autumn. It contains a considerable proportion of living leaves and branches, thus tends to exhibit higher nutrient and lignin content at the same time. The above characteristics enable its treatments to stimulate the growth of soil microbes and enhance their effect of degrading complex aromatic compounds, so as to more effectively remove petroleum contaminants and restore the damaged physicochemical and biological properties of soil (Han et al., 2017; Ding et al., 2016). In addition, because different trees are usually used simultaneously in urban greening, their wastes are often mixed. However, it is still unclear whether and how mixed addition of these greenery wastes could improve (or weaken) their remediation effects on contaminated soil.

The purpose of this study to detect if mixed addition of given greenery wastes could improve their overall remediation effect, and to explore the underlying mechanisms, and finally provide a theoretical basis for further enhancing the efficiency of necrophytoremediation.

2. MATERIALS AND METHODS

2.1. Studied area

The studied area is located in a petroleum production region of Yanjiawan Town, Yan'an City, China (E 109°33'-110°30', N 36°14 '-36°46', 476-1383 m, a.s.l.). The climate here is the warm and semi-humid continental monsoon climate, with an annual average temperature of 10.4℃, an annual average precipitation of 564 mm, and an annual average sunshine duration of 2504.6 h, respectively. The vegetation type here belongs to the forest-steppe zone of the transition from warm temperate deciduous broad-leaved forest to temperate steppe.

The natural vegetation is dominated by *Bothriochloa ischaemum*, *Lespedeza daurica* and *Artemisia* spp. The main soil type is Calcic Cambisols developed from loess parent material, with a bulk density of ~1.1g/cm^3 and an organic matter content of ~5.5 g/kg.

The crude oil production of each well is low and the distribution of wells is relatively wide here, resulting in a large range of historical soil contamination. According to our previous results, the petroleum contents of the contaminated soil here are 11.44-44.72 g/kg (dry soil).

2.2. Sampling and treatments of soil, crude oil, and greenery wastes

In order to avoid the influence of natural attenuation and aging of petroleum caused by background differences, e.g., contamination time and contaminants sources, the soil used for this study was artificially contaminated. In specific, soil samples without any historical contamination were collected from the surface (0-10 cm) of slopes around the wells. Five 1 m × 1m quadrats were randomly established in the slopes, and all the surface soil was collected. After doing this, the soil was passed through a 5 mm sieve for three times, so as to fully mix the soil while removing the animal and plant residues. Subsequently, the treated soil was transported to the laboratory to determine its saturated water holding capacity and actual water content, and then slightly air-dried. At the same time, a part of the soil sample was reserved after air drying, passed through a 1 mm sieve, and used as the uncontaminated control in the following studies. The petroleum (crude oil) used for this study was purchased from local oil field, and the content of saturated, aromatic and non- hydrocarbon substances was 51%, 28% and 21%, respectively.

According to the measured soil petroleum content of 11.44 g/kg in the slightly contaminated area, which has the largest contamination area in study region, the simulated contaminants content was set as 15.00 g /kg in this study. According to the quantity demand of samples, the petroleum and sieved soil were prepared. All petroleum was added to a part of the soil sample and thoroughly mixed by artificial stirring and rubbing. This contaminated soil was then thoroughly mixed with the remaining soil and sieved for five times, to evenly distribute the contaminants in the soil. The prepared contaminated soil was homogenized in the shade for 15 days, so as the acute effects of contaminants on the soil biological and chemical properties could be detected, while the effects of natural attenuation on the actual contaminants content could be eliminated as far as possible. Specifically, the test results showed that the natural attenuation of the contaminants during this period was less than 5%, which was statistically negligible.

Greenery wastes were collected from Baota District, Yan'an City. After the pruning of greenery plants in summer, the wastes of *Platycladus orientalis* (Po), *Picea asperata* (Pa), *Pinus tabuliformis* (Pt), *Acer truncatum* (At), *Ginkgo biloba* (Gb) and *Juniperus formosana* (Jf) were collected. The fresh leaves and twigs of them were selected and dried to constant weight at 65°C. All these materials from each species were ground to pass a 5 mm sieve, respectively, and their contents of nutrient elements and metabolites that related to contaminants degradation were measured (Table 1). In addition, because Po produced much more greenery waste then the other species during tree pruning, part of its powder was mixed with other wastes respectively with the mass ratio of 1:1 for the following experiments.

2.3. Contaminated soil remediation test

Thirty-nine of 500 g-contaminated soil samples were prepared, three of them were directly measured for biological and chemical properties, and used as contamination control; another three of them were directly incubated and used as natural attenuation control;

Table 1. Content of the chemicals of the tested greenery wastes

Greenery wastes	C	N	P	Water-soluble phenols	Soluble saccharide	Terpenes	Flavones	Total organic acids	Amino acids
	/ (g·kg^{-1})							/(mg·kg^{-1})	
Po	436.23 (15.32)a	10.82 (0.52)b	0.95 (0.06)c	17.00 (2.04)c	2.04 (0.24)c	33.66 (8.93)c	37.28 (8.28)c	2.48 (0.07)de	1.73 (0.02)c
Pa	261.76 (9.15)c	13.20 (2.24)b	1.57 (0.23)b	21.72 (0.48)b	4.65 (0.56)a	43.11 (2.32)c	84.81 (2.72)a	11.27 (0.27)b	0.89 (0.15)d
Pt	274.19 (11.88)c	5.76 (0.19)d	0.12 (0.00)d	19.31 (0.51)bc	2.91 (0.28)b	93.91 (7.24)a	60.74 (4.29)b	3.62 (0.36)d	0.81 (0.11)d
At	240.30 (17.72)c	19.36 (2.92)a	1.06 (0.00)c	25.79 (1.29)a	2.96 (0.09)b	60.63 (6.43)b	70.73 (0.96)b	6.62 (0.64)c	7.23 (0.12)a
Gb	426.81 (6.99)b	8.91 (0.05)c	3.48 (0.15)a	11.30 (0.39)d	1.67 (0.12)cd	63.53 (15.96)b	8.02 (1.15)e	15.48 (0.25)a	1.93 (0.08)c
Jf	439.31 (25.76)a	10.37 (0.24)bc	1.57 (0.30)b	3.71 (0.06)e	1.17 (0.09)d	97.22 (1.72)a	13.31 (0.44)d	2.21 (0.18)e	3.69 (0.02)b

Data were represented as average (SE), different letters in the same column indicate significant differences, *P*<0.05.

18 samples were separately mixed with the powder of six types of monospecific greenery waste with a proportion of 2%, and the remaining 15 samples were separately mixed with the powder of five types of mixed wastes with the same waste proportions. At the same time, three samples of uncontaminated soil were taken as the control to determine whether petroleum contamination deteriorated soil biological and chemical properties and the degree of deterioration. All controls and treatments had three independent replicates.

After these, the samples used for natural attenuation control and those treated with greenery waste were moved into polypropylene plastic pots. According to the difference between the measured water content and 50% saturated water capacity, the soil samples were evenly moisturized. Then, using a lid with four of 0.5 cm-diameter air holes was used to cover the pots to control the rapid water evaporation and ensure the normal ventilation for soil microbes, and the pots were incubated continuously at room temperature (20-25°C) for 150 days. During this process, the pots were weighed every two weeks and the soil samples were moisturized to maintain the relatively constant water content.

2.4. Chemical and biological determinations

After the remediation test, all soil samples were retrieved, air-dried in a dark place, and passed through a 0.5 mm sieve to confirm the greenery wastes decomposed totally. Then, these samples were used to determine the contaminants degradation rate and soil biological and chemical properties. The mass remaining of petroleum and its saturated, aromatic and non-hydrocarbon substances of soil was determined by dichloromethane/ultrasonic extraction-column chromatography separation-gravimetric method (Zhu & Tang, 2014). The content of nitrate and ammonium N of soil was determined by potassium chloride solution extraction-indophenol blue colorimetry/ultraviolet spectrophotometry, the content of available P was determined by sodium bicarbonate solution extraction-phosphorus molybdenum blue colorimetry, and the pH value was determined using glass electrode method with the ratio of water to soil of 2.5:1 (Bao, 2000). The activities of soil invertase, urease, alkaline phosphatase and dehydrogenase were determined by colorimetry, while the catalase activity was determined by potassium permanganate titration (Guan, 1986).

2.5. Data processing and statistical analyses

Referring to the basis of previous studies that determining whether the mixed addition of plant residues led to non-additive effects on soil biological and chemical properties (Li et al., 2020), equation (1) was used to calculate the theoretical predicted value of the soil properties obtained after the treatment of mixed greenery wastes (*Pre*), in conditions of the greenery wastes did not affect their remediation effects of each other:

$$Pre=1/2(S_1+S_2) \quad (1)$$

where while S is the measured value of soil properties under the treatments of two types of corresponding monospecific waste.

Student's t-test was employed to find if there was significant difference between the predicted and measured values (C) of the soil properties under the treatment of mixed greenery wastes (α= 0.05). For those showing significant differences, equation (2) was used to calculate the improvement rate (Δ) of the measured values compared with the corresponding predicted values.

$$\Delta=(C-Pre)/Pre\times100\% \quad (2)$$

One-way ANOVA was used to detect the differences of each tested indicator among control and treatments, and the least significant difference (LSD) method was used for the *post hoc* analyses (α= 0.05). In addition, the chemical differences between each two of the greenery wastes was measured by the distance between their corresponding coordinates in the PC1 and PC2 coordinate systems, which was obtained by a principal component analysis (PCA) on all the chemical indices of each type of greenery waste. Finally, redundancy analysis (RDA) was employed to detect the relationships between the chemical characteristics of mixed greenery wastes (including chemical composition and dispersion) and the improvement rate in remediation effect. The above analyses were conducted using SPSS 22.0 and Canoco 5.0, respectively, while all drawings were conducted using Origin Pro 2021 and Canoco 5.0.

3. RESULTS

3.1. Effects of mixed addition of greenery wastes on their efficiency of degrading contaminants

Petroleum and its components exhibited significant natural attenuation within 150 days (Figure 1). Pa, Pt, Gb and Js waste addition significantly promoted the overall degradation of petroleum (P < 0.05). Specifically, all monospecific waste addition significantly promoted the degradation of aromatic hydrocarbons (AHs) and non-hydrocarbon substances (NonHs, P<0.05). Only Pt and Js waste addition significantly promoted the degradation of saturated hydrocarbons (SHs, P<0.05), while Po, Pa and At waste addition exhibited inhibitory effects (P < 0.05).

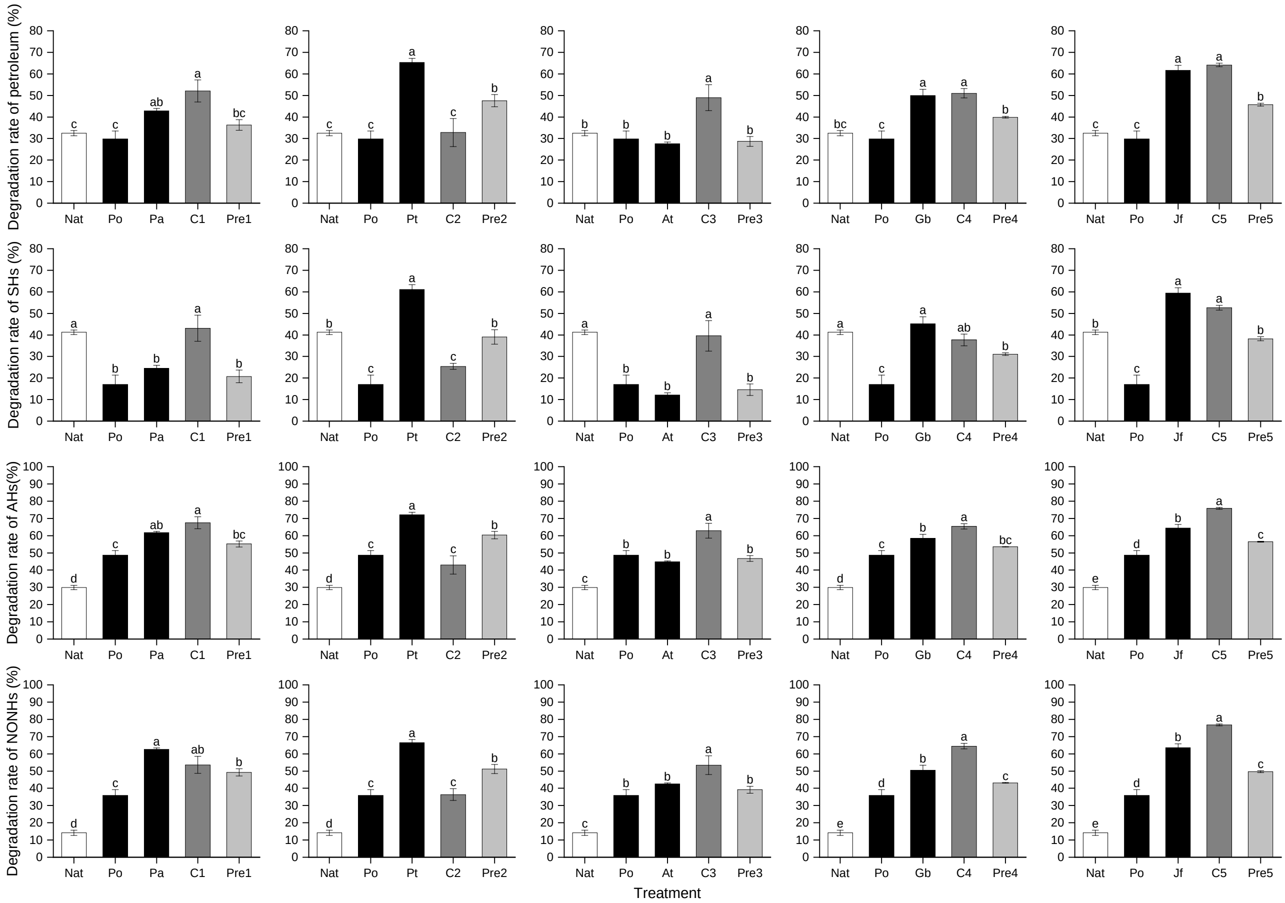

Figure 1. Degradation rate of petroleum, saturated and aromatic hydrocarbons and non-hydrocarbon substances with different treatments

White bars (Nat: natural attenuation) and black bars indicate the values of each indicator obtained in controls and monospecific greenery wastes treated soil, respectively; The dark gray (C) and light gray bars (Pre) indicate the observed and the corresponding predicted values of indicators obtained in mixed waste treated soil. Numbers 1-5 indicate mixing forms including the monospecific greenery wastes in the same subgraphs. Different letters in same subgraph indicate significant differences among treatments, P <0.05, n=3. Po: *Platycladus orientalis*; Pa: *Picea asperata*; Pt: *Pinus tabuliformis*; At: *Acer truncatum*; Gb: *Ginkgo biloba*; Jf: *Juniperus formosana*. The same as below.

Generally, waste mixture addition significantly promoted the degradation of petroleum and its AHs and NonHs components. However, they did not affect the degradation of SHs, while Po-Pt waste mixture addition even caused significant inhibitory effects (P < 0.05). Among them, the mixed addition of Po-Pa, Po-At, Po-Gb and Po-Jf wastes led to significant synergistic effects on the degradation of petroleum and its AHs and NonHs components, while the mixed addition of Po-Pt wastes led to significant antagonistic effects on the degradation of all contaminants (P<0.05).

3.2. Effects of mixed addition of greenery wastes on their efficiency of replenishing soil available nutrients

The contents of nitrate and ammonium N and available P of soil significantly reduced after short-term petroleum contamination (P<0.05, Figure 2). Natural attenuation only recovered the nitrate N content of contaminated soil to a certain extent, but it further reduced the available P content (P<0.05). Except for Pa, all monospecific waste addition significantly reduced soil nitrate N content; Pa waste addition significantly increased soil ammonium N content, while Pt, Gb, and Jf waste addition caused contrast effects. Except for Pt, all types of monospecific waste addition significantly increased soil available P content (P<0.05).

All waste mixtures addition significantly decreased the nitrate N content of contaminated soil (P<0.05). Po-At, Po-Gb and Po-Jf waste mixtures addition significantly increased the ammonium N content of soil, while Po-Pt waste mixtures addition caused the contrast effects (P<0.05). All waste mixtures addition significantly increased available P content of soil (P<0.05). Among them, all forms of mixed addition did not affect their effects of replenishing soil nitrate N (P>0.05); Only the mixed addition of

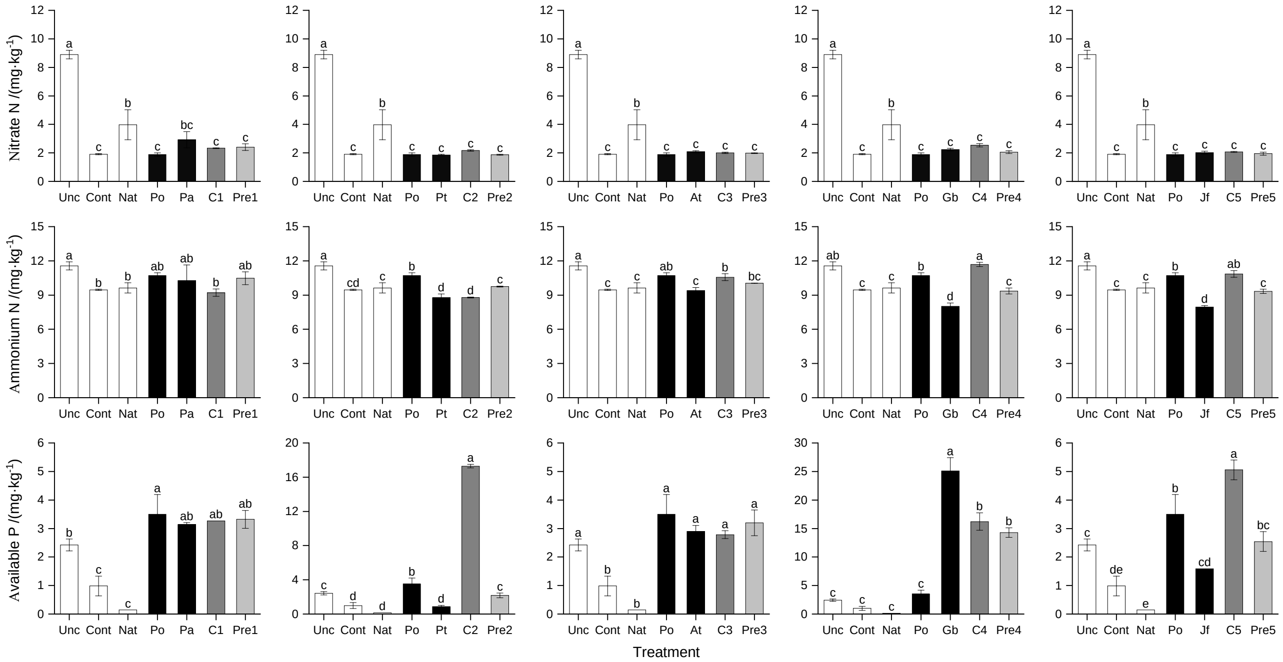

Figure 2. Contents of available nutrient in soil with different treatments
Unc: uncontaminated soil; Cont: Contaminated soil. The same as below

Po-Gb and Po-Jf wastes led to synergistic effects on their effect of replenishing soil ammonium N, while the mixed addition of Po-Pt wastes caused antagonistic effect (*P*<0.05); Only the mixed addition of Po-Pt and Po-Jf wastes caused synergistic effects on their effect of replenishing soil available P (*P*<0.05).

3.3. Effects of mixed addition of greenery wastes on their efficiency of stimulating soil enzymatic activity

Short-term petroleum contamination significantly inhibited soil urease activity (*P*<0.05, Fig. 3), while it significantly increased soil alkaline phosphatase and dehydrogenase activities (*P*<0.05). Natural attenuation significantly inhibited the activities of phosphatase and dehydrogenase, which were significantly increased after contamination (*P*<0.05). It also significantly increased soil catalase activity, which was not affected after contamination (*P*<0.05). Except for Pt and Jf, all monospecific waste addition significantly stimulated soil invertase activity; except for Pa and Pt, all monospecific waste addition significantly stimulated soil phosphatase activity; except for Pt, all monospecific waste addition significantly stimulated soil catalase activity (*P*<0.05), However, all monospecific waste addition significantly stimulated soil urease and dehydrogenase activities (*P*<0.05).

All waste mixtures addition significantly stimulated the activity of the mentioned soil enzymes (*P*<0.05). Among them, the mixed addition of Pa-Pt and Po-Gb wastes led to synergistic effects on their effects of stimulating invertase activity, the mixed addition of Po-Pt, Po-Gb and Po-Jf wastes caused significant synergistic effect on their effects of stimulating urease activity, while the mixed addition of Po-Pt wastes produced antagonistic inhibitory effects (*P*<0.05); The mixed addition of wastes except in form of Po-Jf caused synergistic effects on their effects of stimulating phosphatase activity (*P*<0.05); The mixed addition of Po-Pa wastes led to a synergistic effect on their effects of stimulating catalase activity, while the mixed addition of Po-Jf wastes produced an antagonistic inhibition (*P*<0.05); The mixed addition of wastes except in form of Po-At caused synergistic promotion on their effects of stimulating dehydrogenase activity (*P*<0.05).

3.4. Dominate factors affecting the remediation effects of mixed greenery wastes

Based on the results of RDA (Figure 4A and 4B), higher contents of C, N, terpenoids and amino acids, as well as chemical dispersion, of the greenery waste mixtures were conducive to their effects of stimulating the degradation of petroleum contaminants, while higher contents of soluble saccharides and total water-soluble phenol exhibited adverse effects (Figure 4A). Higher N content of waste mixtures was adverse to their effects of replenishing soil nitrate N and available P, while higher contents of soluble saccharides and total organic acid/ terpenoids of them exhibited contrast effects. Higher N content of waste mixtures was conducive to their effects of stimulating the activity of 5 enzymes, while higher contents of total organic

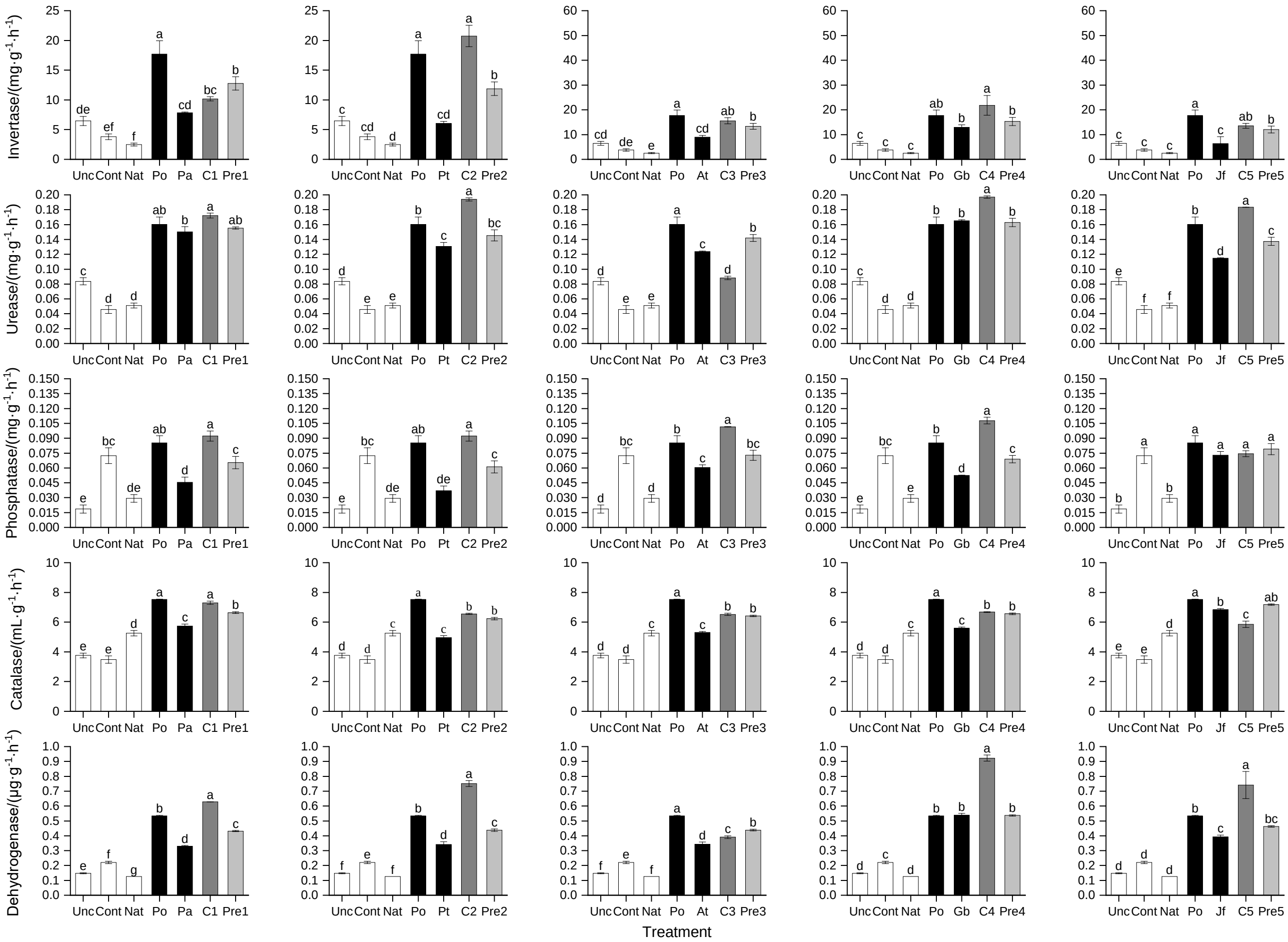

Figure 3. Activity of the crucial enzymes in soil with different treatments

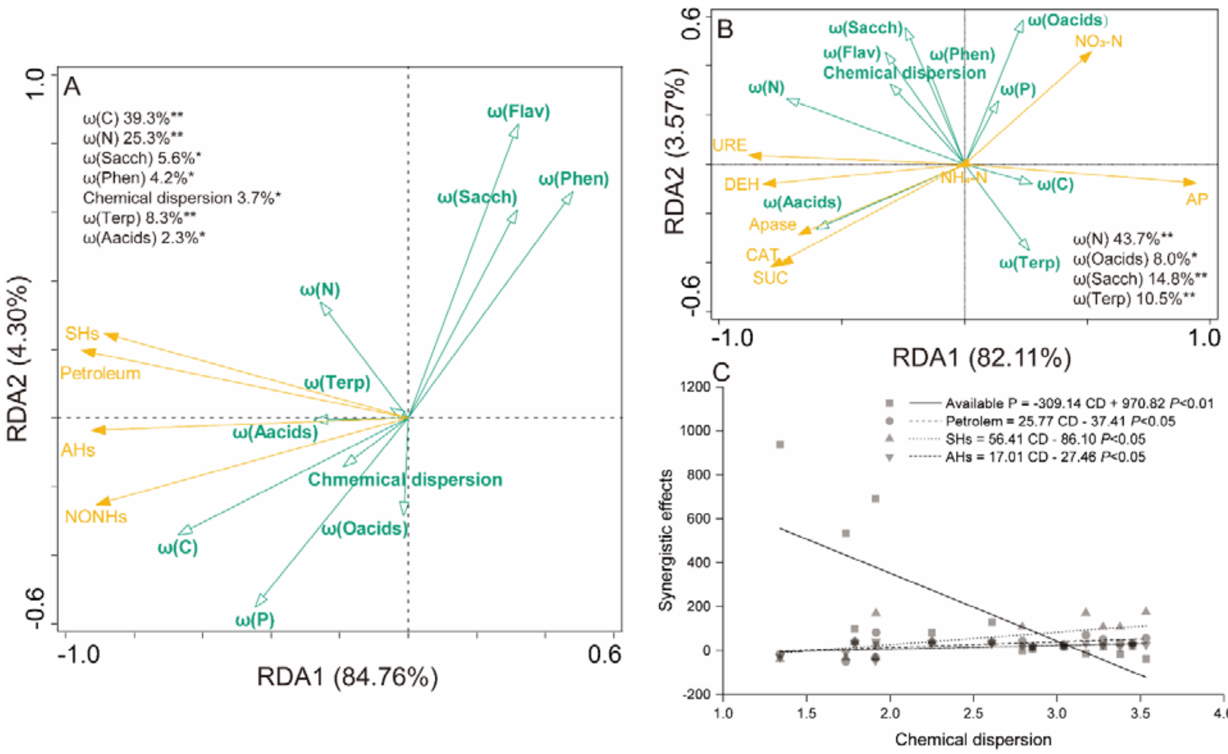

Figure 4. Relationships between the chemical characteristics of mixed greenery wastes and their remediating effects
"ω" in subgraphs A and B indicates the contents of each element or substance in greenery waste mixtures, please see the full names of these abbreviations in Figure 1. Only the significant relationships between the chemical dispersion of mixed wastes and the improvement rate in remediation effect for each soil indicators were shown in subgraph C.

acids and terpenoids led to adverse effects (Figure 4B). In addition, the results of regression analysis (Figure 4C) exhibited that the increase in chemical dispersion of waste mixtures tended to cause synergistic effect on their effects of stimulating the degradation of petroleum and its SHs and AHs components, while it tended to cause antagonistic effect on their effects of replenishing soil available phosphorus ($P<0.05$).

4. DISCUSSIONS AND CONCLUSIONS

4.1. Effects of mixed addition of greenery wastes on their efficiency of degrading contaminants

As expected, except for in the form of Po-Pt, all mixed addition of greenery wastes led to significant non-additive effects, usually synergistic, on the degradation of contaminants. This might be firstly attributed to that all wastes can provide various nutrient elements and metabolites for microbes, because all the metabolites involved in this study were mixtures of multiple compounds. This met the demand of soil microbes for nutrients and was conducive to their rapid reproduction (Chen et al., 2020; Li et al., 2016). At the same time, the screening effect of different organic substances on microbes might adjust their community structure, and significantly improve their species richness and diversity (Chen et al., 2020). The increases in microbial populations might thus promote the decomposition of greenery wastes in turn, so that they could release enough nutrients and degradation-auxiliary substances before the aging of contaminants, and directly promoted their degradation; On the other hand, microbes with greater diversity could promote the degradation of various contaminants and their intermediates by interspecific cooperation (Chen & Yuan, 2012), and the soil function recovery caused by which might be conducive to the contaminant-degrading microbes to continuously obtain nutrients. For instance, the relative abundance of N-cycling related microbes in the contaminated soil exhibited a significant recovery after straw addition treatments (Wu et al., 2012). In addition, many studies have confirmed that the nutrient-rich plant residues usually transfer N to the other plant residues in mixture by hypha, to help their decomposition, and thus improving the overall decomposition rate of mixtures (Kou et al., 2020a). As fungi can connect different substrates across a certain distance (Steffen, 2003), they might also cause nutrient transfer between different waste fragments in this study, accelerate their overall decomposition, and thus promoting their nutrient release, the growth of microbial degraders, and consequently, the degradation of contaminants. In Figure 4C, we found increasing chemical dispersion of mixed wastes favored their synergistic effect on contaminates degradation, which corroborated the mentioned hypothesis. However, our results exhibited that the mixed addition of Po and Pt significantly weakened their effects of degrading contaminants. This might be attributed to that this waste mixture had the lowest chemical dispersion of 1.66, which was significantly lower than those of 2.22-3.13 in other mixtures. Consequently, the similarity of chemical traits of Po and Pt might cause more competition among microbes, inhibit their growth, and thus weaken their ability of degrading contaminants. In addition, Po waste contained limited N and P nutrients, while Pt waste was even more lacking in these nutrients. Therefore, the nutrient transfer between these wastes might inhibit the decomposition of each other, leading to limitations in their nutrient release and in their effects of stimulating the microbial degradation of contaminants (Zhang et al., 2019).

Notably, the actual promoting effects on the degradation of contaminants of waste mixtures were still dominated by their contents of C, N, and their availability, such as the content of N in the form of free amino acids, which were in line with the previous studies (Koshlaf et al., 2020; Ding et al., 2016; Yu et al., 2015). Because there usually are sufficient contaminant-degrading microbial species in soil (Huang et al., 2019), while their growth was limited as the unbalanced stoichiometric ratios of petroleum-derived C and key nutrient elements such as N and P. The greenery wastes have much higher content of nutrient elements (Table 1) and lower ratios in C/N and C/P (12-48 or 123-2285, calculated from Table 1) than those of contaminants, which meant that the wastes exhibited better lability then contaminants, and they could decompose quickly and provide amounts of N and P to microbes. This would stimulate the microbial activities, and consequently, promote the degradation of contaminants before their aging (Koshlaf et al., 2016b; Koshlaf et al., 2019). In addition, previous studies indicated that the secondary metabolites, such as terpenoids, from greenery waste may accelerate the degradation of relatively stable structures, such as aromatic rings in contaminants, by co-metabolism pathways (Koshlaf et al., 2016b; Koshlaf et al., 2019; Zhen et al., 2020). Likewise, the organic acids, phenolic substances and soluble saccharides can also promote the desorption of contaminants from soil particles, and consequently, the degradation of them. However, the promoting effects of the former one type of metabolites were not detected, while the other two types of metabolites exhibited adverse effects in this study. These might be attributed to that phenolic substances, such as water-soluble phenols and flavones, might inhibit the growth of microbes and the activity of their nutrient-harvesting enzymes (Chomel et al., 2016) at higher concentrations. However, when the contents of labile carbon sources, such as soluble saccharides, in greenery wastes were excessive, it might specialize the soil indigenous microbes to communities utilizing labile and nutrient-rich substances (Chomel et al., 2015). Therefore, the addition of given wastes might decrease the degradation rate of some contaminants.

4.2. Effects of mixed addition of greenery wastes on their efficiency of recovering the damaged biological and chemical properties

Mixed addition of greenery wastes also caused significant non-additive effects on their efficiency of restoring the damaged biological and chemical properties of soil. It rarely affected their effects of replenishing soil available nutrients. Because all wastes would be completely decomposed in the conditions of this study, thus the non-additive effect of mixed decomposition could not affect the total amount of nutrients released from the wastes. However, we did observe synergistic/antagonistic effects caused by mixed addition of greenery waste with several forms on their effects of replenishing soil available nutrients. Because the simultaneous addition of two types of waste would lead to the alterations in the biomass, community structure and functional characteristics of soil microbes relative to the monospecific waste treatments (Zhang et al., 2021). It might consequently affect the nutrient transformation process in the soil. In addition, the mixed addition of wastes might affect their stimulating effects on the activity of enzymes that related to soil nutrient cycling, which would further support our hypothesis mentioned before, because most of soil enzymes are secreted by microbes.

Compared with soil nutrient characteristics, soil enzyme activity was more commonly affected by the mixed addition of greenery wastes. The reason might be that the species composition, metabolic characteristics and environmental stress of soil microbes were significantly changed (Zhang et al., 2021) due to the changes in the nutrient supply rate, carbon sources diversity and contaminant degradation rate during the mixed decomposition of wastes. These might enhance or weaken their resource allocation in certain metabolic pathways, such as carbon source and nutrient acquisition, contaminant degradation or anti-active oxygen capacity (Yang et al., 2021). Hence, the activities of corresponding enzymes, such as invertase, urease and phosphatase, dehydrogenase and catalase, were consequently changed. Besides, the non-additive effects of mixed addition of wastes on the degradation of contaminants significantly changed their contents in soil, and consequently, the activities of urease, dehydrogenase and phosphatase. Because urease was usually sensitive to the toxicity of contaminants, excessive carbon sources from the contaminants might force microbes to secrete more phosphatase, while aromatic contaminants might induce dehydrogenase secretion of microbes as well (Thavamani et al., 2012; Zhao et al., 2017). Finally, the mixed decomposition of greenery wastes also caused non-additive effects on their release of metabolites. For instance, during the mixed decomposition of coniferous and broad-leaved litters, the release of total phenols and condensed tannins of the mixture generally deviates from their theoretical predicted values (Qin et al., 2018), leading to changes in their chemical stress to microbes and enzymes. These non-additive effects, the complex composition of the same type of secondary metabolites in different wastes, and the different ecological effects among specific compounds might jointly affect the soil enzyme activity, resulting in complex potential effects on the soil enzyme activities caused by mixed addition of greenery wastes.

However, the effects of mixed wastes of replenishing soil nutrient and stimulating soil enzymatic acting also dominated by their contents of N, organic acids, soluble saccharides and terpenoids. In which, the N content of mixed wastes exhibited significant negative relationship with their effects of replenishing soil nitrate N and available P. This might be attributed to that the N-rich waste would promote the degradation of petroleum contaminants to a great extent, which would also consume a large amount of N and P, leading to apparent decrease in their contents of soil (Yu et al., 2015; Chen et al., 2017). Certainly, the demand of microbes for P is much lower than that for N (the ratio of C, N and P required for the degradation of contaminants is about 100:10:1), thus the content of available P in soil might still significantly increase due to the replenishment of greenery wastes. On the contrary, the effects of mixed wastes of replenishing soil nutrient positively correlated with their content of soluble saccharides, organic acids, and terpenoids, because their acidic decomposition products are conducive to activate the N and P in soil. The increase in N content of mixed wastes favored their effects of stimulating soil enzymatic activities, this might be attributed to that these wastes might release more N, which provided sufficient N sources for microbes to secret enzymes. In Fig. 4B, the positive relationships between the amino acids content of waste and the soil enzymatic activities also corroborated the mentioned hypothesis. Correspondingly, waste-derived organic acids might decrease soil pH, while excessive terpenoids would inhibit the growth of soil microbes and their enzyme secretion, or directly deposit the enzymes (Chomel et al., 2016). Therefore, the increases in the content of these substances were adverse to the effects of stimulating soil enzymatic activity of mixed greenery wastes.

4.3. Other issues that need to be addressed

In this study, we mainly detected the potential enhancement of mixed addition of greenery wastes on their remediation effects on petroleum contaminated soil, this phenomenon and the underlying mechanism might provide a feasible approach to strengthen the effects of necrophytoremediation. However, it should be noted that all the decomposition of the greenery wastes used in this study and their remediation of contaminated soil were conducted in lab conditions. When this approach was applied in natural conditions, the decomposition of greenery waste might be hindered by the relative harsher hydrothermal conditions, and consequently, weaken their actual remediation effects. Therefore, it is necessary to choose suitable application time (like summer, which has favorable precipitation and temperature), or other approaches accelerating the decomposition of wastes should be applied together when treating contaminated soil using mixed greenery wastes. In addition, although the loess soil (Calcic Cambisols) used in this study has favorable ability of holding the contaminants in the surface layer (mainly the layer of 0-20 cm, unpublished data) like other clayey-textured soil (Lăcătușu et al., 2021), however, the alterations in soil humus or carbon content caused by greenery waste treatments, which closely related to the soil porosity, might indirectly affect the leaching of contaminants and their migration to underground water, while the complex terrain condition might also affect this process (Paltineanu et al., 2022). Therefore, how these affect the actual remediation effects of mixed greenery wastes should be addressed in the following studies.

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