Effects of root exudates of woody species on the soil anti-erodibility in the rhizosphere in a karst region, China

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Introduction: The rhizospheres, the most active interfaces between plants and soils, play a central role in a long-term maintenance of the biosphere. The anti-erodibility of soils (AES) regulated by the root exudates is crucial to the stability of the rhizospheres. However, scientists still remain unclear regarding the key organic matter in the root exudates to affect the AES and interspecific variation. Methods: We used an incubation of soils to test the effects of the root exudates from 8 woody plant species on a change in soil aggregation, and identified the organic matter in these root exudates with gas chromatograph-mass spectrometer (GC-MS) and biochemical methods. Furthermore, the relationships between the organic matter in the exudates and the AES in the rhizosperes of the 34 additional tree species were analyzed. Results: The water-stable aggregates of the soils incubated with the root exudates increased by 15-50% on average compared with controls, and the interspecific differences were significant. The root exudates included hundreds of specific organic matter, in which hydrocarbon, total sugar, total amino acids and phenolic compounds were crucial to the AES. These types of the matter could explain about 20-75% of the variation in total effects of the root exudates on the AES quantified by aggregate status, degree of aggregation, dispersion ratio and dispersion coefficient. Discussion: Effects of the root exudates on the AES and the interspecific variation are as important as those of root density, litters and vegetation covers. A range of studies have explored the effects of root density, litters, vegetation covers and types on the AES, but little attention has been given to the effects of the root exudates on the AES. Different plants secrete the different relative contents of the organic matter resulting in the variation of the effects of the root exudates on the AES. Our study quantified the causal relationships between the root exudates and the AES from modeling experiments in laboratory to actual effects in the field, and indicated the interspecific variation of the AES and the organic matter in the root exudates. Conclusions: The study recognized more

organic compounds in the exudates related to the AES. These results can enhance the understanding of the stability of the soils in a slope and be applied to ecosystem restoration.

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Short title: the anti-erodibility of soils and the root exudates

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ABSTRACT

Introduction: The rhizospheres, the most active interfaces between plants and soils, play a central role in a long-term maintenance of the biosphere. The anti-erodibility of soils (AES) regulated by the root exudates is crucial to the stability of the rhizospheres. However, scientists still remain unclear regarding the key organic matter in the root exudates to affect the AES and interspecific variation.

Methods: We used an incubation of soils to test the effects of the root exudates from 8 woody plant species on a change in soil aggregation, and identified the organic matter in these root exudates with gas chromatograph-mass spectrometer (GC-MS) and biochemical methods. Furthermore, the relationships between the organic matter in the exudates and the AES in the rhizosperes of the 34 additional tree species were analyzed.

Results: The water-stable aggregates of the soils incubated with the root exudates increased by 15-50% on average compared with controls, and the interspecific differences were significant. The root exudates included hundreds of specific organic matter, in which hydrocarbon, total sugar, total amino acids and phenolic compounds were crucial to the AES. These types of the matter could explain about 20-75% of the variation in total effects of the root exudates on the AES quantified by aggregate status, degree of aggregation, dispersion ratio and dispersion coefficient.

Discussion: Effects of the root exudates on the AES and the interspecific variation are as important as those of root density, litters and vegetation covers. A range of studies have explored the effects of root density, litters, vegetation covers and types on the AES, but little attention has been given to the effects of the root exudates on the AES. Different plants secrete the different relative contents of the organic matter resulting in the variation of the effects of the root exudates on the AES. Our study quantified the causal relationships between the root exudates and the AES from modeling experiments in laboratory to actual effects in the field, and indicated the interspecific variation of the AES and the organic matter in the root exudates.

Conclusions: The study recognized more organic compounds in the exudates related to the AES. These results can enhance the understanding of the stability of the soils in a slope and be applied to ecosystem restoration.

INTRODUCTION

The rhizosphere, a term firstly used by Hiltner, is a zone of soil surrounding the root which is affected by the zone and its size differs spatially and temporally depending on the factors considered, ranging from a fraction of a millimetre for microbial populations and immobile nutrients, to tens of millimetres for mobile nutrients and exudates released from roots (Gregory, 2006). The rhizosphere is different from the bulk soil due to a range of biological, chemical and physical processes that occur as a consequence of root growth, release of exudates and rhizodeposition (Kandeler et al., 2002; Marschner & Baumann, 2003; Hinsinger et al., 2005). As seeds germinate and roots grow through the soil, the release of the exudates from the roots begins to change all these processes, such as soil particles sticking together to form soil aggregates, organic anion increasing nutrient availability, signaling molecules selectively inducing the multiplication of microbes, and directly provide the driving forces for the development of soil structure (Whipps, 2001; Walker et al, 2003). The formation and stability of the rhizospheres have a great contribution to the stability of a slope, carbon sequestration and the maintenance of the biosphere, and thus the rhizospheres have partially made the Earth get a contrasting trait different from other planets (Walker et al., 2003; Kuzyakov, Hill, Jones,2007; Vannoppen et al., 2015).

The root exudates, that are considered an influencing factor matching root density, litters and vegetation cover, have a significant effect on the AES (Fattet et al., 2011; Vannoppen et al., 2015). The root exudates play several roles in directly and indirectly strengthening the AES: (1) The adhesive properties of the root exudates bind soil particles together to enhance the formation of the water-stable aggregates (Bronick & Lal, 2005; De Baets et al., 2008); (2) The release of the root exudates is a continual source of the organic matter which will improve the soil structure, referring to the size, shape and arrangement of solids and voids, continuity of pores and voids, and their capacity to retain and transmit fluids and organic and inorganic substances (Lal,1991); (3) The aggregate formation and stability are indirectly influenced by microorganisms which feed on the root exudates and produce hypha and polysaccharides to bind soil particles together (Andrade et al., 1998). The strengthened AES increases resistance to erosions of raindrops, surface runoff, concentrated flow and seepage flow at the plant-soil interface (Vannoppena et al., 2015).

Many studies have dealt with these roles. For example, Tisdall & Oades (1982) found that the water-stable aggregates (>0.25mm) were dependent on the root exudates, and fungal hyphae, and the stability of micro-aggregates was determined by the contents of persistent organo-mineral complexes and transient polysaccharides. Czarnes et al. (2000) mixed a bacterial xanthan,and an analogue of root mucilage (polygalacturonic acid) with soils to simulate the adhesive effects of the root exudates, suggesting that xanthan and polygalacturonic acid increased the tensile strength of the soils. Subsequently, the rhizosphere soils had been observed to contain larger pores than the bulk soils (Whalleyet al., 2005). In a silty soil, the soils adhered to maize roots had a greater strength of aggregation (450–500 kPa) than that no adhered (410–420 kPa, Czarnes, Dexter, Bartoli, 2000). Many studies also indicated that mycorrhizal hyphae was implicated in the adhesion of soil particles to roots, together with root hairs, immature xylem vessels and the mucilage from roots, resulting in the formation of the rhizosheaths (Amellal et al., 1998; McCully, 1999; Young & Crawford, 2004). In addition, microbial biomass carbon, hot-water soluble carbohydrate carbon and soil organic carbon were assumed to be the compositions of the root exudates leading to the formation of soil aggregates, and experimental results indicated that the chemical bonding of these compositions accounted for 14.7% of the variation in macro-aggregates (>0.212 mm), while the physical binding of root systems accounted for 39.0% (Jastrow et al., 1998; Wang et al., 2014).

Overall, these studies advance the field primarily by the following aspects: (1) The root exudates were stimulated with analogues, and the mixtures of soil samples and the root exudates stimulated were incubated to test the effects of the root exudates on the AES (Morel et al., 1991); (2) The root exudates collected from 1 or 2 annual crop plants were mixed with the soil samples, and the mixtures were incubated to identify the effects of the root exudates on the AES; (3) The effects of the root exudates on the AES were separated theoretically or experimentally from other factors such as root density, litters and vegetation cover. However, it still remains unclear what types of the organic matter in the root exudates in the rhizospheres of different woody plants are crucial to the AES. The interspecific variation of the organic matter and the AES in the rhizospheres of these woody plants are also unknown. Moreover, while the effects of the root exudates had been identified in laboratory, some caution is also required in extrapolating to field conditions (Gregory, 2006). Additionally, there is a strong karstification in karst regions, where concealed erosion is a primary way of soil erosion (Wang et al., 2014). The concealed erosion is a phenomenon of vertical movement of soil particles with a seepage flow but little surface runoff occurs. Understanding of the relationships between the AES and the root exudates has a special significance for controls of the concealed erosion. However, little attention has given to these relationships.

In the study, we conducted the incubation experiments and extrapolation experiments to clarify the above-mentioned aspects (Figure 1).We primarily focus on the following questions: 1) when the soil subsamples from a soil sample are respectively incubated with the root exudates from eight tree species, how the water-stable aggregates, micro-aggregates, MWD (mean weight diameter) and GMD (geometry mean diameter) of these soil subsamples response to these root exudates? Which organic matter compounds in the root exudates are more closely related to the MWD and GMD? 2) When the tree species tested increased to 34 species plus previous 8 plants, what variation will be indicated from the test results of the organic matter in the root exudates and the comprehensive indices of the AES (including aggregation status, degree of aggregation, dispersion ratio and dispersion coefficient) in the rhizospere soils? How these comprehensive indices of the AES response to the contents of the key organic matter?

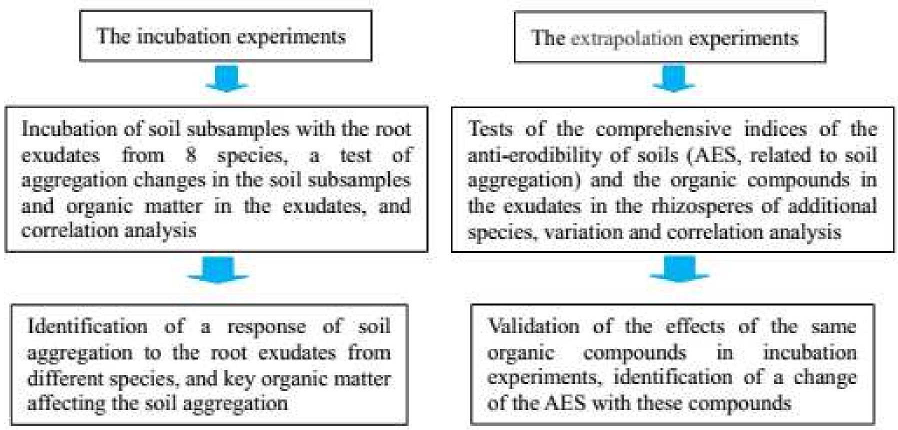

Figure 1 the flowchart of the study

MATERIALS AND METHODS

Extraction of the root exudates

We selected eight of the typical tree species to extract the root exudates in a karst forest of Qianling mountains (106º41´-106º42´E, 26º17´-26º22´N, 1100-1396m E) in Guiyang city, China. These species included *Carpinus pubescens, Cladrastis platycarpa, Zanthoxylum planispinum Sieb.et Zucc., Ligustrum lucidum, Itea yunnanensis, Cinnamomum glanduliferum, Cyclobalanopsis gracilis,* and *Platycarya longipes*. We further determined three sample trees for each species based on similarity in an individual growth, and no diseases, pests and anthropogenic disturbance impacting the individual growth. Then, litters, humus layers and soils under the canopy of each sample tree were removed. After finding the living fibrous roots, we peeled external (> 1cm thickness) soils around the fibrous roots and collected inner (0-1 cm thickness) soils (no less than 500g). The subsamples, which just equaled 60g dried soils, were taken from the 500g soil samples and put in a wide mouth bottle. The root exudates in the subsamples were extracted with 200 ml of aether under a condition of oscillation at 20°C for 1 hour. Then, the mixture in the wide mouth bottle was filtrated and the filtrate was condensed at 20°C with a rotary evaporator. Lastly, the condensed filtrate was diluted to 10 ml, i.e., the mother liquid of the root exudates. The soil samples remained were sifted through 2-mm and 0.25-mm

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sieves after open-air drying for a week, which were used to test the organic matter in the root exudates. All field experiments in the study had been approved by Administration Bureau of Two Lakes and One Reservoir in Guiyang City.

Incubation of soils and tests of the AES

About 2 kg of soil samples was collected from a depth of 0-20cm at a site in an evergreen broadleaf forest (elevation: 1220 m) in Guiyang city. Soil type was a rendzina soil. After open-air drying, the soil samples were sifted through a 2-mm sieve. Then, we respectively weighed three of 30 g of the soil samples, put them in three 100 ml conical flasks and successively added 1, 2 and 3 times of the volume (10ml) of the mother liquid from one tree species to these conical flasks. Different volumes of the mother liquid were replicated 3 times. The controls were conducted by adding the same volume of the distilled water to three 100 ml conical flasks with 30 g of the soil samples. Then, we adjusted the C/N of the mixtures in all these conical flasks to 10 with KNO_3 solution. The water content of the mixtures was also necessarily adjusted to about 60% of field moisture capacity. Subsequently, all these conical flasks were closed with rubber stoppers and incubated at 25°Cin the illuminating incubators for 8 hours every day till the twenty-fifth day. Then, the water-stable aggregates and micro-aggregates of the soils incubated were tested. The water-stable aggregates were tested by the wet screening method with an aggregate analyzer, which included the five particle diameters: >2 mm, 2-1mm, 1-0.5 mm, 0.5-0.25 mm and <0.25 mm. The micro-aggregates were measured by pipette method, which included 0.25-0.05mm, 0.05-0.02mm, 0.02-0.002 mm and <0.002mm. The MWD (equation 1) and GMD (equation 2) were calculated.

$$\mathrm{MWD} = \frac{\left(\sum_{i=1}^{n} \overline{X}_i W_i\right)}{\sum_{i=1}^{n} W_i} \qquad [1]$$

$$\mathrm{GMD} = \exp\left(\frac{\sum_{i=1}^{n} W_i \ln \overline{X}_i}{\sum_{i=1}^{n} W_i}\right) \qquad [2]$$

Where $\overline{x}_i$ was the mean diameter (mm) of the aggregates within a particle-size range; W_i was the ratio of the weight (g) of the aggregates within the particle-size range to the total weight (g) of the soil sample.

GC-MS analysis of the organic matter and tests of the biological active matter

We weighted 40 g of the soil samples from the rhizosperes of each tree species, sieved it all to pass through a 40 mesh sieve and put it in a 500ml conical flask with a stopper. Then, 150ml of dichloromethane was decanted into the conical flask. The conical flask was corked by a stopper and continually oscillated for 1 hour. Subsequently, the mixture in the conical flask was extracted for 20 minutes with ultrasonic waves and filtrated. The residue was collected and placed into another 500ml conical flask. The same steps as above were conducted to extract the root exudates in the residue. The filtrates from two times of the extraction were mixed, concentrated for 20 minutes with a rotary evaporator and dissolved with 5 ml aether that had passed through a 0.45 μm filter membrane. Lastly, the mixed liquid was absorbed into a sterile centrifuge tube for GC-MS analysis.

GC-MS analysis of the organic matter was performed on an HP 6890 gas chromatograph equipped with an Agilent MSD 5975C mass spectrometer (Agilent Technologies). Chromatographic column was AB-5MS 5% Phenyl-95% DiMethylpolysiloxane (30 m × 0.25 mm × 0.25μm) elastic quartz capillary column. The temperature in the vaporization chamber was maintained at 250°C. Highly pure Helium was used as the carrier gas, with a flow rate of 1.0 ml min^{-1}. The inlet pressure was at 7.62 psi. Split ratio was 20:1. A solvent delay time was set at 1.5 min (Müller et al., 2008; Hutzler et al., 2014). Identification of the organic matter and their relative contents was conducted with a mass spectrometry data system. Specifically, the different peaks of the total ion spectrum were firstly compared with the standard spectrum in the Nist 05 and Wiley 275 databases to determine what volatile constituents existed in the root exudates. Then, the peak area normalization method was used to measure a relative mass fraction of the volatile constituents.

We tested the biological active matter, including total sugar, total amino acids, phenolic compounds and free amino acid in the rhizospere soils, respectively using anthracenone colorimetry (Abdelhamid et al., 2013), tri-ketone colorimetric method (Song et al., 2009) and Folin-ciocalteu colorimetry (Song et al., 2009; Faujdar et al., 2012).

Tests and analysis of the additional plant species

We selected other 34 tree species (different from those eight tree species) in the karst forests around Guiyang city to validate the effects of the root exudates. Specifically, we used the same methods as above to collect the rhizosphere soils of three sample trees for each species, extract the root exudates and identify the contents of the organic matter with the GC-MS (but only 13 tree species showed the satisfactory flow diagrams). The biological active matter was also detected with the biochemical methods. The indices of the AES: aggregate status, degree of aggregation, dispersion ratio and dispersion coefficient of the rhizospere soils from the 34 species and from the previous 8 plants were quantified with the equation (3)-(6) (Wang et al., 2014). Lastly, the relationships between these indices of the AES and the organic matter in the root exudates were analyzed.

Aggregate status (%) = (the micro-aggregate at a ＞ 50 μm particle diameter, %)-(soil mechanical components at a ＞50μm particle diameter, %) [3]

$$\text{Degree of aggregation (\%)} = \frac{\text{Aggregate status} \times 100}{\text{The micro - aggregates at a} > 50\mu\text{m particel diameter}} \quad [4]$$

$$\text{Dispersion ratio (\%)} = \frac{\text{The micro - aggregate at a} < 50\mu\text{m particle diameter} \times 100}{\text{Soil mechanical components at a} < 50\mu\text{m particle diameter}} \quad [5]$$

$$\text{Dispersion coefficient (\%)} = \frac{\text{The micro - aggregate at a} < 2\mu\text{m particle diameter} \times 100}{\text{Soil mechanical components at a} < 2\mu\text{m particle diameter}} \quad [6]$$

Date analysis

T-test was used to measure the differences between the water-stable aggregates, micro-aggregates, MWD and GWD respectively and controls, i.e. test of single population. Specifically, we used the formula $t=(\bar{\mu}-\mu_0)\frac{\sqrt{n-1}}{s}$, where $\bar{\mu}$ and μ_0 were the mean of the indices of the anti-erodibility of the soil samples incubated respectively by the root exudates and the distilled water (controls); s is the standard deviation ; n was the number of the samples. A coefficient of variation (CV) was used to test the interspecific variation of the individual indices of the AES (F-test and T-test could not be applied here). The CV (%) = $s \times 100/\bar{\mu}$. When the $CV \geq 30\%$, it was statistically defined that there was significantly different among plant species; when the $CV < 30\%$, a significant level was determined by CV_u. The CV_u was an upper confidence limit of the CV and when the $CV < CV_u$, there was not significant variation among these plant species in statistic. Here, the CV_u $=\{(X_{1-\alpha}^{2}(n-1)[1+CV^2(n-1)/n]\}/[(n-1) CV^2]$, where $X_{1-\alpha}^{2}(n-1)$ was gained by searching the quantiles of Chi squared distribution when free degree = n-1 and probability = $1-\alpha$ (Standardization Administration of PRC, 2009).

The interspecific differences of the relative contents of the organic matter identified by GC-MS and the biological active matter were also tested with the CV. A comparison of the AES between the rhizospheres soils and non-rhizospheres soils was conducted by t-test of a double-population. The relationships between the indices of the AES and the organic compounds were described by Pearson`s correlation coefficients. The significant levels were also tested by t-test.

RESULTS

Effects of the root exudates on the AES

The water-stable aggregates (>2mm and 2-1mm) of the soils incubated with 1~3 times of the mother liquid of the root exudates increased except for few of the soil samples compared to controls (Table 1). The increases averaged 15.52% and 21.39% (1x), 13.33% and 35.58% (2x) and 19.25% and 40.65% (3x) at the two particle diameters, respectively. There were relatively higher water-stable aggregates (>2mm and 2-1mm) in the rhizosphere soils of *C. platycarpa*, *C.gracilis, I.yunnanensis* and *P.longipesresulted* than other plants. However, the incubation of the soils with 1~ 3 times of the mother liquid resulted in the decreases of the water-stable aggregates (<0.25mm) by 41.3% (1x), 51.34% (2x) and 58.30% (3x) (Table 1). It was noted that the water-stable aggregates of the soils incubated with 2~3 times of the mother liquid did not always show a higher percentage than one time. T-test indicated significantly different between the water-stable aggregates (>2mm, 2-1mm and <0.25mm) and the controls. The water-stable aggregates at a 0.5-0.25mm particle diameter showed a relatively small change compared with the controls, although t-test was also significantly different. The water-stable aggregates at a 1-0.5 mm particle diameter did not indicate obviously different between the soils incubated and the controls (Table 1). CV and CV_u indicated that the water-stable aggregates at all particle diameters were significantly different among these tree species, and comparatively, the aggregates at a 1-0.5mm or 0.5-0.25mm diameter had a greater difference than other particle diameters (Table 1).

Table 1 Percentage compositions of the water-stable aggregates in the soils samples incubated with the root exudates from the eight plant species

Tree species	Concentrations	The water-stable aggregates at different particle diameters (%)				
		>2mm	2-1mm	1-0.5mm	0.5-0.25mm	<0.25mm
Controls	0	14.26±1.07	16.95±1.49	28.13±1.07	7.10±0.28	33.56±0.86
Carpinus pubescens	1x*	18.18±0.20	17.30±0.30	25.87±1.70	8.10±0.78	30.56±1.54
	2x	14.94±0.46	22.23±0.91	29.09±1.74	6.99±0.85	26.76±1.73
	3x	17.41±1.34	20.84±0.83	27.17±0.80	8.16±0.42	26.42±1.66
Cladrastis platycarpa	1x	14.93±1.87	22.57±2.08	26.67±0.94	8.35±0.50	27.48±1.10
	2x	20.53±1.11	23.91±1.61	23.66±3.02	7.00±0.92	24.91±1.50
	3x	18.81±0.49	23.07±1.34	24.97±1.75	7.64±0.44	25.50±1.38
Zanthoxylum Planispinum Sieb.et Zucc.	1x	14.12±0.66	25.33±1.36	25.05±1.75	8.23±1.37	27.28±0.66
	2x	15.82±0.64	20.32±2.35	28.27±2.09	6.85±0.79	28.76±0.68
	3x	15.90±1.74	22.50±2.63	28.15±0.72	7.50±0.15	25.95±1.07
Ligustrum lucidum	1x	17.89±0.29	17.42 ±2.14	28.43±0.75	9.19±0.79	27.06±2.31
	2x	17.17±0.90	15.40±1.27	29.88 ±2.29	9.11±0.38	28.45±1.10
	3x	16.42±1.14	23.13±1.06	26.34±0.91	8.11±1.07	26.00±1.47
Itea yunnanensis	1x	16.68±1.58	19.85±1.26	27.79±1.33	10.38±0.96	25.30±1.00
	2x	14.05±0.38	22.40±0.71	27.85±1.48	10.08 ±1.27	25.62±1.30
	3x	18.28±2.03	25.76 ±1.50	24.48±0.82	8.70 ±0.96	22.77±2.10
Cinnamomum glanduliferum	1x	13.99±1.40	20.75±2.40	30.24 ±1.29	8.39 ±0.49	26.63±0.92

	2x	13.97±0.30	24.63±0.61	27.79±0.34	6.89 ±0.32	26.72±0.84
	3x	14.78±0.86	23.01±1.13	26.81±0.92	9.91±0.48	25.48±1.59
Cyclobalanopsis gracilis	1x	19.11±3.64	18.26±1.96	27.87±2.35	6.57±0.16	28.19±1.21
	2x	19.41±0.73	19.96±1.82	28.25±1.55	6.64±1.03	25.75±1.75
	3x	19.95±1.68	20.20±1.84	28.70±0.62	7.05±0.52	24.10±0.60
Platycarya longipes	1x	16.87±1.06	18.52±0.64	27.41±0.51	8.35±0.81	28.85±0.75
	2x	13.38±0.90	27.34±2.42	28.29±1.17	8.03±0.81	22.95±0.91
	3x	14.45±1.91	23.45±2.53	29.21±1.51	7.13±0.70	25.76±0.88
T-test	1x	T=3.03, p<0.01	T=2.89,p<0.025	T=1.19, p>0.05	T=3.33,p<0.01	T=9.93, p<0.005
	2x	T=1.90,p<0.05	T=3.75,p<0.005	T=0.36,p>0.05	T=1.26,p>0.05	T=10.26,p<0.005
	3x	T=3.71, p<0.005	T=9.06 ,p<0.005	T=1.81,p>0.05	T=2.61, p<0.025	T=18.18,p<0.005
Coefficients of variaiton (CV,%)	CV of 1x	11.74	13.99	5.83	12.66	5.68
	CV_u	1.29	1.28	1.30	1.29	1.30
	CV of 2x	16.42	16.27	6.60	16.47	7.20
	CV_u	1.28	1.28	1.30	1.28	1.29
	CV of 3x	11.50	7.45	6.28	11.74	4.80
	CV_u	1.29	1.29	1.30	1.29	1.31

*1x, 2x and 3x represent the treatments incubated by 1, 2, and 3 times of the mother liquid of the root exudates. The same below.

The MWD of the soils incubated with 1~3 times of the mother liquid increased by an average of 8.58%, 11.34% and 13.52% compared with the controls (Fig.2A). T-test indicated significantly different (1x: t=11.58, p<0.005, n=8; 2x: t=5.86, p<0.005, n=8; 3x: t=10.03, p<0.005, n=8). The MWDs of the soils incubated with the root exudates extracted from the rhizosphere soils of *C.platycarpa*, *I. yunnanensis* and *C. gracilis* were relatively greater than other tree species. The GMD of the soils increased by 10.16%,13.87% and 16.41% respectively compared with the controls (Fig.2B). The increasing rates were higher than the MWD. T-test also indicated significantly different (1x: t=13.13, p<0.005, n=8; 2x: t=7.09, p<0.005, n=8; 3x: t=11.08, p<0.005, n=8). The GMD of the soils incubated with the root exudates from the rhizospheres of *C.platycarpa*, *I. yunnanensis* and *C. gracilis* were also greater than other plant species (Fig.2B). However, the values of the MWD and GMD did not always increase with increasing the volume of the mother liquid.

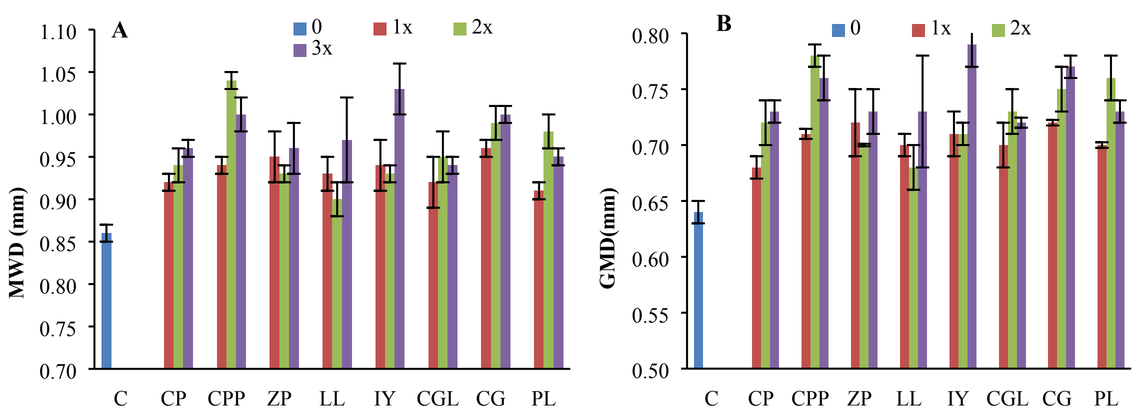

Figure 2 MWD (mean weight diameter, A) and GMD (geometry mean diameter, B) of the soils incubated with the root exudates from the eight tree species. C: Control; CP: *C.pubescens*; CPP: *C.platycarpa*; ZP: *Z. planispinum*

Sieb.et Zucc.; LL: *L.lucidum*; IY: *I.yunnanensis*; CGL: *C.glanduliferum*; CG: *C.gracilis*; PL: *P. longipes*.

Compared with the controls, the micro-aggregates (0.05-0.02mm) of the soils incubated with 1~3 times of the mother liquid decreased by 46.42% (1x), 54.72% (2x) and 36.01% (3x), respectively. However, the micro-aggregates (<0.002mm) only decreased by 10.70%, 15.34% and 21.59% (Table 2). Conversely, the micro-aggregates (0.02-0.002mm) indicated a great increase and the increasing rates were respectively 129.85%, 135.68% and 157.1%. T-test indicted that there were not significant differences between the micro-aggregates at a 2-0.25mm or 0.25-0.05mm diameter and the controls (Table 2). Comparatively, the differences were more significant between the micro-aggregates at a 0.05-0.02mm, 0.02-0.002mm or <0.002mm diameter and the controls. Based on *CV* and $CV_{u,}$ the micro-aggregates at different particle diameters were also significantly different among these tree species except for the micro-aggregate (at a 2-0.25 mm particle diameter) of the soils incubated with 2 times of the mother liquid. The *CV* was relatively great for the micro-aggregates at a 0.05-0.02 mm or 0.02-0.002 mm particle diameter.

Biological active matter and organic matter in root exudates for incubation of soils

The content of the total sugar was highest and lowest respectively in the rhizosphere soils of *C. pubescens* and *P. longipes* (Table 3). The highest and lowest contents of the total amino acids occurred in the rhizosphere soils of *C. platycarpa, and L. lucidum and I. yunnanensis.* However, there were the highest contents of the phenolic compound and free amino acid in the rhizosphere soils of *C. platycarpa* and *I. yunnanensis*, respectively. The contents of all the biological active matter were closely related to the MWD and GMD in the rhizospere soils of these eight plants and the total amino acids showed the highest significance. We found that there were high contents of free amino acid or total amino acid in the rhizospere soils of *C. platycarpa*, *I.yunnanensis C.gracilis* and *P.longipes*. The rhizospere soils of the four plants just contained high water-stable aggregates (>2mm and 2-1mm, Table 1) and showed great MWD and GMD (Fig.1).

Table 2 Percentage components of the micro-aggregates of the soil samples incubated with the root exudates from the eight plant species

Tree species	Concentrations	The micro-aggregates at different particle diameters (%)				
		2-0.25mm	0.25-0.05mm	0.05-0.02mm	0.02-0.002mm	<0.002mm
Controls	0	71.87±1.94	12.45±0.84	5.72±1.07	2.27±0.52	7.69±1.00
Carpinus pubescens	1x	71.35±2.09	12.29±1.57	4.92±0.48	4.44±1.25	7.00±0.60
	2x	73.82±1.23	12.62±1.37	2.52±0.79	4.60±0.85	6.44±1.08
	3x	70.11±1.65	13.69±2.41	3.72±0.44	6.08±0.27	6.40±1.03
Cladrastis platycarpa	1x	72.28±2.17	13.92±1.26	1.44±0.53	5.52±1.34	6.84±0.47
	2x	71.53±0.88	13.11±0.61	2.60±0.08	6.12±0.92	6.64±0.75
	3x	70.94±2.22	10.98±0.92	5.04±1.32	7.24±1.65	5.80±1.04
Zanthoxylum Planispinum Sieb.et Zucc.	1x	69.97±2.71	13.99±0.65	4.08±0.65	5.16±1.16	6.80±1.43
	2x	74.16±2.25	12.04±2.24	3.52±1.61	4.88±1.54	5.40±0.74
	3x	70.73±2.53	12.55±1.70	5.00±0.64	5.56±0.53	6.16±1.03
Ligustrum lucidum	1x	74.51±4.12	11.01±1.95	2.16±0.36	6.48±0.70	5.84±1.32
	2x	72.15±2.16	13.13±0.47	3.12±0.42	5.44±0.82	6.16±0.82
	3x	72.57±0.91	11.51±1.20	3.60±0.79	6.60±0.98	5.72±0.96
Itea yunnanensis	1x	75.35±2.63	10.37±0.30	3.56±0.76	4.22±0.52	6.50±1.25

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	2x	73.78±3.13	12.54±2.01	3.24±0.26	4.36±1.16	6.08±1.31
	3x	74.09±0.91	11.07±1.10	2.88±0.73	5.76±0.33	6.20±1.46
Cinnamomum glanduliferum	1x	72.60±1.93	10.61±0.69	3.36±0.30	6.64±1.88	6.80±0.55
	2x	73.29±1.47	14.35±1.62	1.40±0.23	3.68±0.57	7.28±0.58
	3x	73.10±2.72	12.78±2.00	3.20±0.23	4.64±1.33	6.28±0.90
Cyclobalanopsis gracilis	1x	73.16±3.34	12.20±2.16	2.12±1.18	5.08±1.06	7.44±1.02
	2x	73.28±1.79	10.40±0.91	2.16±1.11	7.40±1.60	6.76±1.32
	3x	73.61±1.09	11.31±0.97	2.08±0.52	6.52±1.58	6.48±0.28
Platycarya longipes	1x	73.51±2.44	11.69±0.76	2.88±0.47	4.20±0.61	7.72±2.14
	2x	73.83±4.25	10.37±1.85	2.16±0.72	6.32±1.53	7.32±0.50
	3x	75.53±3.16	11.11±1.71	3.76±0.58	4.40±1.02	5.20±0.86
T-test	1x	T=1.50, p>0.05	T=0.84, p>0.05	T=6.13, p<0.005	T=8.18, p<0.005	T=3.82, p<0.005
	2x	T=3.91, p<0.005	T=0.25, p>0.05	T=11.95, p<0.005	T=6.72, p<0.005	T=4.87, p<0.005
	3x	T=1.01, p>0.05	T=1.52 , p>0.05	T=5.44, p<0.005	T=9.71, p<0.005	T=10.25,p<0.005
CV	*CV* of 1x	2.35	11.54	37.38	18.28	8.30
	CV_u	1.41	1.29	N/A*	1.28	1.29
	CV of 2x	**1.26**	11.11	26.75	22.67	9.84
	CV_u	**1.68**	1.29	1.28	1.28	1.29
	CV of 3x	2.58	8.45	27.39	16.67	7.11
	CV_u	1.39	1.29	1.28	1.28	1.30

*N/A shows the value of *CV* great enough (*CV*>30%) and it does not need to be tested with the CV_u.

The organic matter in the root exudates identified by the GC-MS primarily included hydrocarbon, amide, alcohol, phenolic ether, aldehyde, acid, ketone, ester, and others (low concentrations of matter, Table 3). Each type also included many specific compounds (Supplemental file 1). These types of the organic matter took up more than 80% of the total organic matter in the root exudates except for *C. glanduliferum* and *C. gracilis*. The hydrocarbon had the highest percentages among all these organic matter. The specific matter of the hydrocarbon

Table 3 Contents of biological active matter, relative contents of the organic matter identified by GC-MS and their correlations with the MWD and GMD

Tree species	Total soluble sugar (g/kg)	Total amino acids (g/kg)	Phenolic compound (g/kg)	Free amino acid (mg/kg)
Carpinus pubescens	1.64	0.91	3.38	9.13
Cladrastis platycarpa	1.24	1.04	3.49	14.24
Zanthoxylum planispinum Sieb.et Zucc.	1.31	0.78	2.43	2.88
Ligustrum lucidum	1.11	0.24	3.39	12.38
Itea yunnanensis	1.49	0.24	2.71	17.97
Cinnamomum glanduliferum	1.51	0.36	2.76	12.10
Cyclobalanopsis	1.11	0.84	3.08	3.28

gracilis				
Platycarya longipes	1.05	0.80	2.32	10.65
Correlation coefficients	0.41*; 0.48*	0.50**;0.55**	0.52**;0.48*	0.39*; 0.44*
n	24	24	24	24

Tree species	Hydrocarbon (%)	Amide (%)	Alcohol (%)	Phenolic ether (%)	Aldehyde (%)	Acid (%)	Ketone （%）	Ester （%）	Other (%)	Total (%)
Carpinus pubescens	37.09 (35)	8.61(3)	15.36(12)	1.45(1)	0.72(2)	4.35(2)	4.93	6.87(3)	5.26(7)	84.62
Cladrastis platycarpa	31.85(21)	11.91(3)	3.10 (3)	9.73 (3)	1.73(4)	0.00(0)	1.14(1)	27.51(4)	1.72(4)	88.70
Zanthoxylum planispinum Sieb.et Zucc.	36.72(40)	1.25(2)	12.24(11)	10.63(4)	1.07(3)	3.70(2)	2.11(4)	13.75(5)	2.94(5)	84.40
Ligustrum lucidum	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Itea yunnanensis	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cinnamomum glanduliferum	26.11(32)	1.04(3)	13.99(13)	20.57(2)	2.05(3)	1.99(4)	4.30(6)	6.15(7)	1.29(2)	77.47
Cyclobalanopsis gracilis	31.29(50)	7.64(4)	9.37(11)	2.04(4)	1.68(3)	0.17(1)	8.30(8)	5.42(5)	3.00(9)	68.91
Platycarya longipes	37.08(35)	5.22(3)	22.11(11)	7.16(2)	0.47(1)	0.87(1)	1.38(4)	4.58(4)	2.67(6)	81.53
Correlation coefficients(r)	0.39; 0.48*	0.67**; 0.64**	-0.12; 0.08	0.02; 0.08	0.48*; 0.51*	-0.31;- 0.28	0.27; 0.33	0.57*; 0.49*	0.16; 0.20	0.42; 0.51*
n	18	18	18	18	18	18	18	18	18	18

The values in the parenthesis are the number of the specific organic matter identified by GC-MS. N/A shows no identified due to the unsatisfactory flow diagrams. *,** or *** represents a significance at a 95, 99 or 99.9 confidence level, respectively.

was also most. The amides, phenolic ether, alcohols and esters in a percentage were relatively lower than the hydrocarbon. Only four types of the organic matter were closely related to the MWD and GMD. The amide, aldehyde and ester showed a higher correlation than other organic matter. In the rhizospere soils of *C. platycarp* and *C.gracilis*, there were relatively high contents of the amide and ester. Correspondingly, the rhizospere soils of the two plants contained high water-stable aggregates (>2mm and 2-1mm, Table 1), and showed the great MWD or GMD (Fig.1)

The AES and the root exudates in the rhizosphere soils of the additional plants

Aggregation status and degree of aggregation of the rhizosphere soils of the additional plant species were greater than the non-rhizosphere soils (Table 4). Conversely, dispersion ratio and dispersion coefficient were smaller than the non-rhizosphere soils. Paired t-test indicated that the indices of the AES in the rhizosphere and non-rhizosphere soils were significantly different except for the degree of aggregation. The aggregation status, degree of aggregation, dispersion ratio and dispersion coefficient showed high variation among the additional plant species because the *CVs* of the four indices of the AES were greater than their CV_u. The *CVs* of the dispersion ratio and dispersion coefficient were also more than 30% (Table 5).

Table 4 A comparison of the AES of the rhizosphere and non-rhizosphere soils of the additional plant species

Indices of the AES	Position	Average (%)	N	Standard deviation	t	P
Aggregation status	R	51.17	42	14.47	3.014	0.0044<0.01
	B	48.04	42	14.47		
Degree of aggregation	R	70.61	42	15.84	1.484	0.1455>0.05
	B	69.04	42	16.25		
Dispersion ratio	R	36.29	42	13.25	-3.343	0.0018<0.01
	B	40.09	42	13.23		
Dispersion coefficient	R	25.06	42	10.04	-2.024	0.04957<0.05
	B	28.12	42	12.95		

R: the rhizosphere soils; B: non-rhizosphere soils

Table 5 Statistical quantities of the indices of the AES, the contents of the biological active matter and the relative contents of the organic matters identified by GC-MS respectively in the rhizosphere soils of the additional plants

Indices of the AES	Aggregation status	Degree of aggregation	Dispersion ratio	Dispersion coefficient
Maximum (%)	71.09	85.77	71.10	44.69
Minimum (%)	21.51	37.35	16.93	10.45
Mean (μ,%)	51.17	70.61	36.29	25.06
Variance (σ)	209.49	250.89	175.69	100.80
Standard deviation (s)	14.47	15.84	13.25	10.04
Coefficients of variation (CV)	28.29	22.43	36.52	40.06
CV_u	1.17	1.17	1.16	1.16
n*	42	42	42	42

Biological active matters	Total soluble sugar	Total amino acids	Phenolic compound	Free amino acid
Maximum (g/kg)	2.26	1.04	3.81	0.01835
Minimum (g/kg)	0.27	0.17	0.81	0.00286
Mean (μ, g/kg)	1.06	0.56	2.52	0.01010
Variance (σ)	0.23	0.09	0.82	0.00002
Standard deviation (s)	0.48	0.29	0.90	0.00397
Coefficients of variation (CV)	45.75	52.32	35.83	39.33
CV_u	1.16	1.16	1.16	1.16
n	42	42	42	42

Parameters	Hydrocarbon	Amides	Alcohols	Phenolic ether	Aldehyde	Acids	Ketone	Esters	Others	Total
Maximum (%)	49.66	11.91	33.55	20.57	2.61	21.47	11.70	27.51	5.26	92.01
Minimum (%)	18.98	0.44	3.10	1.12	0.23	0.16	1.04	4.12	0.55	67.28
Mean (μ, %)	37.27	4.36	14.58	6.35	1.25	3.16	4.23	8.69	2.78	82.11
Variance (σ)	65.43	10.54	47.57	40.71	0.41	23.83	7.62	31.22	1.95	50.45
Standard deviation (s)	8.09	3.25	6.90	6.38	0.64	4.88	2.76	5.59	1.40	7.10
Coefficients of variation (CV)	21.70	74.41	47.30	100.47	50.92	154.70	65.31	64.27	50.28	8.65
CV_u	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.21
n	19	18	19	19	19	17	19	19	19	19

n: the number of plant species.

The variation of the biological active matter was smaller than the organic matter detected by CG-MS based on the CV. Other statistical quantities: variance, standard deviation, mean, maximum and minimum also reflected the variation. On average, the total soluble sugar and phenolic compound were higher in the contents than the total amino acids in the rhizosphere soils of the additional plant species (Table 5). Free amino acid was lowest. All CVs of the contents of the biological active matter were not only greater than CV_u, but also more than 30%, indicating a significantly interspecific difference. The relative content of the hydrocarbon was highest among all nine organic matter identified by GC-MS. The alcohols and esters were respectively ranked second and third in the relative contents. The CV and CV_u also showed that there were significant differences in relative contents of these organic matter compounds among the additional 19 plant species because most of the CVs were far greater than 30%. Only

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the hydrocarbon and the total in a relative content were a bit lower than 30%. However, they both were greater than CV_u (Table 5). Comparatively, the interspecific variation of the organic matter identified by GC-MS was more significant than the biological active matter (Table 5).

Most of the organic matter (identified by GC-MS) in the root exudates from the rhizosphere soils of the additional plant species were not significantly correlated in the relative contents with the indices of the AES (Table 6). Only the hydrocarbon showed a significant correlation with two indices of the AES, i.e., the aggregation status and dispersion ratio. If a 90% confidence level was considered as a weak significant correlation, the relative content of the phenolic ether was also significant. However, almost all of the biological active matter was highly correlative with the indices of the AES (Table 6).

Table 6 Coefficients of the correlations between the indices of the AES and the relative contents of the organic matter identified by GC-MS and the biological active matter in the rhizosphere soils of the additional plant species

Types of organic matter		Aggregation status			Degree of aggregation			Dispersion ratio			Dispersion coefficient		
		r	p	n	r	p	n	r	p	n	r	p	n
Organic matter identified by GC-MS	Hydrocarbon	**0.47**	<0.05	19	0.31	>0.10	19	**-0.46**	<0.05	19	-0.03	>0.10	19
	Amides	-0.30	>0.10	19	-0.23	>0.10	19	0.29	>0.10	19	-0.2	>0.10	19
	Alcohols	-0.04	>0.05	19	-0.27	>0.10	19	-0.06	>0.10	19	-0.22	>0.10	19
	Phenolic ether	-0.05	>0.05	19	0.15	>0.10	19	0.12	>0.10	19	**0.39**	**<0.10**	**19**
	Aldehyde	-0.13	>0.05	19	0.05	>0.10	19	0.16	>0.10	19	-0.11	>0.10	19
	Acids	0.22	>0.05	19	0.08	>0.10	19	-0.23	>0.10	19	0.01	>0.10	19
	Ketone	-0.18	>0.05	19	-0.21	>0.10	19	0.13	>0.10	19	-0.06	>0.10	19
	Esters	0.22	>0.05	19	0.16	>0.10	19	-0.19	>0.10	19	0.01	>0.10	19
	Others	0.25	>0.05	19	0.24	>0.10	19	-0.23	>0.10	19	-0.2	>0.10	19
Biological active matter	Total sugar	**0.75**	<0.001	42	**0.71**	<0.001	42	**-0.63**	<0.001	42	**-0.27**	**<0.10**	**42**
	Total amino acids	**0.62**	<0.001	42	**0.57**	<0.001	42	**-0.57**	<0.001	42	**-0.28**	**<0.10**	**42**
	Phenolic compound	**0.80**	<0.001	42	**0.87**	<0.001	42	**-0.58**	<0.001	42	-0.32	<0.05*	42
	Free amino acid	0.13	>0.10	42	0.14	>0.10	19	-0.07	>0.10	19	-0.18	>0.10	19

The values in bold type represent significant relationships at a 90%, 95%, 99% or 99.9 confidence level respectively, when p <0.10, 0.05, 0.01, or 0.001.

The indices of the AES indicated different changes with the contents of the organic matter significantly correlated with the AES in Table 6 (Fig. 3). The aggregation status presented an increase with an increasing content of the hydrocarbon but the dispersion ratio presented a significant decrease (Fig.3A). The values of the dispersion ratio showed a bit scattered with the contents of the phenolic ether, but the dispersion ratio increased, indicating the negative effects of the phenolic ether on the AES (Fig.3B). The indices of the AES indicated relatively high regularity with the contents of the biological active matter (Fig.3C-E). Specifically, both the aggregation status and the degree of aggregate presented logarithmic or linear growths with the contents of the biological active matter. However, the dispersion ratio and coefficient presented a logarithmic decrease or

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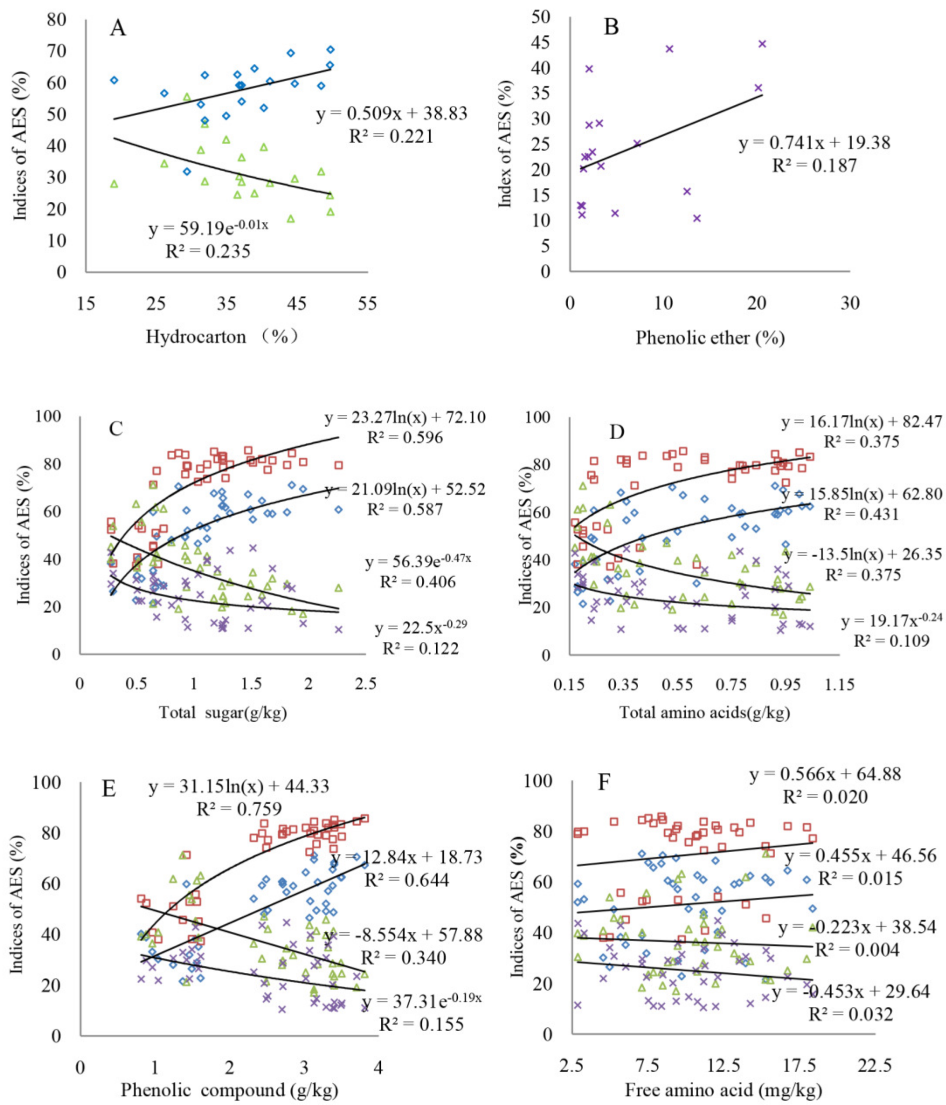

Figure 3 Changes of the indices of the AES with the contents of the key organic matter. A: Hydrocarbon; B: Phenolic ether; C: Total sugar; D: Total amino acids; E: Phenolic compound; F: Free amino acid. In all figures, the triangular symbols: dispersion ratio; the square symbols: degree of aggregation; the diamond symbols: aggregation status; the multiplication sign: dispersion coefficient. The regression models with R^2>0.20 showed the significance at a 95% confidence level (p<0.05) based on goodness of fit test. The number of observation in all figures was the same as in Table 6.

change in power function. These organic matter compounds explained 20-76% of the variation in total effects of the root exudates on the AES based on different R^2. The phenlic compound had the highest explanation power for the aggregation status. It was noted that although the Fig.3F was used to describe the relationships between

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the contents of the free amino acid and the indices of the AES, it could also represent the variable characteristics of other no significantly relative indices of the AES with the contents of the organic matter in Table 6.

DISCUSSION

The direct effects of the root exudates on the AES are often tested by an incubation of the mixtures of soil samples and the root exudates from few annual plants (Song et al., 2009a, 2009b). In-situ experiments are also conducted by the exclusion of the effects of root systems, plant cover and litters to quantify the effects of the root exudates (Wang et al., 2014). Our study was expanded to a collection of the root exudates from eight woody plant species and an incubation experiment to identify the responses of the water-stable aggregates, micro-aggregates, MWD and MGD of the soil samples to the different root exudates. Results indicated that the water-stable aggregates (>2mm and 2-1mm), MWD and MGD increased (Table 1). Conversely, the micro-aggregates (0.05-0.02mm and <0.002mm, Table 2) and the water-stable aggregates (<0.25mm, Table 1) obviously declined. The increases of the water-stable aggregates (>2mm and 2-1mm) ranged from 15.52% to 40.65% on average and MWD and MGD from 8.58%-16.41% compared with the controls. However, the increases were smaller than the previous studies in which the root exudates were simulated using analogues of the root exudates (Traoré et al., 2000) and collected from soybean and maize (Song et al., 2009a, 2009b). This was because the soils incubated in the study was a Rendzina soil, in which, both coarse silt (0.02-0.05mm) and smaller soil particles only occupied 17.34%, far smaller than 84.2% and 59% in the soils incubated respectively taken from a Luvisol (Traoré et al., 2000) and a black soil (Song et al., 2009a, 2009b), which was obviously unfavorable to adhering soil particles to form the water-stable aggregates.

The AES in the rhizospheres of the additional plant species in the extrapolation experiments, quantified with the aggregation status, degree of aggregation, dispersion ratio and dispersion coefficient, were different from the non-rhizospheres (Table 4 and supplemental file 5). These four indices in the rhizosphere soils also indicated highly different among these plant species (Table 5 and supplemental file 6). The interspecific differences were clearly greater than those in the incubation experiments based on the *CV* and CV_u (Table 1, 2 and 5). Previous studies have not given attention to the interspecific differences regarding the woody plants enhancing the AES through their root exudates and this study clarified these by *CV* (McCully, 1999; Czarnes et al., 2000b; Whalley et al., 2005; Young & Crawford, 2004; Wang et al., 2014). Previous studies also suggested that the impact of the root exudates on soil physical properties and structure was still to be deciphered and thus series of structured repacked samples were incubated with a daily input of the artificial root exudates (Milleret et al., 2009; Kohler-Milleret et al., 2013). Results indicated that the root exudates increased microbial activity and aggregate stability and decreased the small-diameter structural porosity. The study enhances the understanding of the impacts of the root exudates on soil physical properties through the responses of the AES described by the different indices to the root exudates, and interspecific differences.

The root exudates are defined as diffusible compounds, in which free sugars, amino acids and organic acids have been widely recognized to not only have adhesive effects (Jones, Nguyen and Finlay, 2009), but also have a variety of the biological active effects (Whipps, 2001; Song et al., 2009a, 2009b). The biological active matter is needed by soil microbes and the growths of these soil microbes can result in rich myceliums promoting the formation of the water-stable aggregates (Whipps, 2001). In the study, we tested the concentrations of four types of the biological active matter in the root exudates from eight plant species. We further used the GC-MS to identify the specific organic compounds and analyzed the correlations of the organic matter with the AES (Morel et al., 1987; Gessa & Deiana, 1990; Albalasmeh and Ghezzehei, 2014). The organic matter included hydrocarbon, amides, alcohols, phenolic, aldehyde, acids, ketone, esters, and other (small-concentration matters). They took up about 80% of the organic matter in the root exudates, in which the hydrocarbon was highest in relative contents. The biological active matter and hydrocarbon, mide, aldehyde, ester detected by GC-MS primarily decided the changes of the MWD and GWD (Table 3).

To further validate the effects of the root exudates in the incubation experiments, we measured the organic matter and their contents in the root exudates extracted from the rhizosphere soils of the additional plant species. The number of the organic matter and interspecific variation in relative contents were beyond our expectations (Supplemental file 1 and 2). The contents of the biological active matter showed smaller interspecific variation than the organic matter identified by GC-MS (Table 5 and supplemental file 6). The comprehensive indices of the AES, i.e, aggregation status, degree of aggregates, dispersion ratio and coefficients in the rhizosphere soils of the additional plant species were primarily related to the hydrocarbon, amides, phenolic ether, total sugar, total amino acids and phenolic compound (Table 6 and supplemental file 7). These organic compounds were crucial to the AES, which was similar to the results of the incubation experiments. It was noted that most of the organic matter detected by GC-MS were not significantly associated with these comprehensive indices of the AES. These organic compounds may be the hormone-like compounds of low molecular fractions to affect the growth, nutrient uptakes of other plants and microbes, and allelopathic effects, indirect effects on the AES (Nardi et al., 2005). Further regression analysis indicated that the key organic matter determined by correlation analysis could explain 20-76% of the variation in total effects of the root exudates on the AES (Fig. 3).

CONCLUSIONS

The water-stable aggregates, MWD and GMD of the soils incubated with the root exudates significantly increase. Most of the micro-aggregates and the small water-stable aggregates decrease. The root exudates from *C. platycarpa*, *C.gracilis* and *I.yunnanensis* and *P.longipesresulted* resulted in a relatively higher increase of the water-stable aggregates (>2mm and 2-1mm),WMD and GMD than other plants. In the root exudates, there are hundreds of organic matter compounds. Total sugar, total amino acids, phenolic compound, hydrocarbon, amides and phenolic ether were crucial to the changes of soil aggregation and the AES. The organic matter detected by GC-MS is great in interspecific difference compared with the biological active matter. The organic matter affecting the AES in the extrapolation experiments is similar to that in the incubation experiments, but the biological active matter indicates higher correlation with the AES. The changes of the indices of the AES with the contents of the key organic matter present different forms.

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