

Assaying the potential autotoxins and microbial community associated with *Rehmannia glutinosa* replant problems based on its ‘autotoxic circle’

Bao Zhang · Xuanzhen Li · Fengqing Wang · Mingjie Li · Junyi Zhang · Li Gu · Liuji Zhang · Wanqian Tu · Zhongyi Zhang

Received: 14 January 2016 / Accepted: 4 April 2016 / Published online: 23 April 2016
© The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract

Aims This study attempted to elucidate the underlying correlations among replant problems of *Rehmannia glutinosa*, the autotoxins and microbes within its root-zone soil.

Methods Different root-zone soils of *R. glutinosa* were collected to identify the potential autotoxins, to quantify

the phenolic acids’ concentration and to analyse the microbial community composition. Bioassay was conducted to assess the autotoxic potential of these samples and candidate autotoxins.

Results *R. glutinosa* autotoxicity was presented within a 20 cm radius of the plant. Concentrations of the phenolic acids were significantly higher within this range. Twenty compounds and 17 microbes emerged in this range after the *R. glutinosa* cultivation. Further seedling test showed that four selected compounds possessed conspicuous autotoxic activity and a synergistic effect was observed. Moreover, one potential beneficial fungi (99 % similar with *Arthrobotryx oligospora*) and one potential pathogens (100 % similar with *Acremonium sclerotigenum*) were identified.

Conclusions Our work defined the ‘autotoxic circle’ of *R. glutinosa* and identified the corresponding autotoxins and microbes. We suggest that *R. glutinosa* has various autotoxins and that these autotoxins and specific microbes deserve further investigation to unravel their interaction with *R. glutinosa* in replant problems’ occurrence.

Bao Zhang and Xuanzhen Li contributed equally to this work.

Responsible Editor: Azim U. Mallik.

B. Zhang · Z. Zhang
Key Laboratory of Crop Ecology and Molecular Physiology
(Fujian Agriculture and Forestry University), Fujian Province
University, Fuzhou 350002, PR China

B. Zhang · M. Li · L. Gu · Z. Zhang (✉)
College of Crop Science, Fujian Agriculture and Forestry
University, Fuzhou 350002, PR China
e-mail: zyzhang@fafu.edu.cn

X. Li
College of Forestry, Henan Agricultural University,
Zhengzhou 450002, PR China

F. Wang
College of Agronomy, Henan Agricultural University,
Zhengzhou 450002, PR China

J. Zhang
College of Chemical Engineering, Huaqiao University,
Xiamen 362021, PR China

L. Zhang · W. Tu
Henan Province Chinese Medicine Research Institute,
Zhengzhou 450004, PR China

Keywords *Rehmannia glutinosa* Libosch · Replant
problems · Autotoxins · Microbes

Abbreviations

TCM	Traditional Chinese medicine
HPLC	High-performance liquid chromatography
GC-MS	Gas chromatography-mass spectrometer

PCR-DGGE	Polymerase chain reaction-denaturing gradient gel electrophoresis
CS	Close soil
IS	Intermediate soil
DS	Distant soil
IR	Inhibition rate
KI	Kovats index
MF	Molecular formula
SPSS	Statistical Analysis System Program
SE	Standard error
GAP	Good agricultural practices
GMP	Good manufacturing practices
NIST	National Institute of Standards and Technology
NCBI	National Center of Biotechnology Information

Introduction

Rehmannia glutinosa is a member of Scrophulariaceae family, and it is one of the 50 basic herbal medicines in traditional Chinese medicine (TCM). However, the replant problems of *R. glutinosa* extremely restrict its production. Fields used for *R. glutinosa* cultivation are typically replanted every 15–20 years (Yang et al. 2011b). Jiaozuo City, Henan Province, is the geo-authentic production area of *R. glutinosa* (34° 48' N to 35° 30' N, 112° 02' E to 113° 38' E) (Yang et al. 2011b). Because of the lack of availability of fields in Jiaozuo, *R. glutinosa* is replanted in the same location at intervals of less than 15 years or outside the Jiaozuo area to meet market demand. All of these factors result in a vicious circle that is characterized by a lower tuber yield, worse product quality and higher disease incidence. Therefore, understanding the underlying mechanism of *R. glutinosa* replant problems is urgent.

The inhibitory effect of allelochemicals is regarded as the primary factor inducing replant problems (Muller 1969; Singh et al. 2010; Zhu et al. 2014). According to the previous studies, the occurrence of phenolic acids were frequently reported and regarded as one class of important secondary metabolites possess strong allelopathic activity (Li et al. 2010; Muscolo and Sidari 2006; Olofsdotter et al. 2002). What is noteworthy is that the secondary metabolism of plants presents unique diversity and richness and results in more than 200,000 secondary

products, and the phenolic acids are only part of the products of various secondary metabolic pathways (Dixon and Strack 2003; Seigler 1998; Weston et al. 2012). Thus, lots of potential allelochemicals should be identified to explore the plants' allelopathy. In recent years, increasing compounds (like ketones, alkaloids, terpenes) were reported as potential allelochemicals of other plants (Guo et al. 2015; Kato-Noguchi et al. 2011; Ma et al. 2009). Interestingly, the growth inhibitory activity of some potential allelochemicals was much greater than that of phenolic acids (Kato-Noguchi et al. 2012), and some compounds presented a synergistic effect when exhibiting their allelopathic effect (Al Harun et al. 2015; Zhang et al. 2012; Zuo et al. 2015). In previous studies, the polar compounds in ethyl acetate extraction of *R. glutinosa* leaves and fibrous roots have been preliminary analysed for the identification of allelochemicals (Li et al. 2012; Wang et al. 2010). However, the potential autotoxins left in the soil after *R. glutinosa* cultivation are still unknown. Thus, a relative integrated identification of potential autotoxins of *R. glutinosa* is necessary and meaningful in understanding the mechanism of its autotoxicity.

Plant-microbe interactions can positively or negatively influence plant growth through a variety of mechanisms, and root exudates are one of the most important chemical signals in the determination of whether an interaction will be benign or harmful (Bais et al. 2006). Generally, allelochemicals are regarded as the distinctive components of root exudates and somewhat influence the microbial community (Bertin et al. 2003). According to a recent report of *Pseudostellaria heterophylla* replant problems, the accumulated autotoxins in rhizosphere soil increased the harmful microorganisms and decreased beneficial microorganisms, resulting in an imbalance of microbial community structure and the degradation of soil ecological function (Lin et al. 2015). The previous studies found that the structural and functional diversity of soil microbial communities in newly *R. glutinosa* planted soil were distinctly different from consecutively mono-cultivated soils, and the fungal community decreased with the increase of mono-cultivated years (Wu et al. 2011; Zhang et al. 2011). However, information on microbial community distribution within root-zone soil of *R. glutinosa* and the influence of *R. glutinosa* cultivation on microbes are still unclear. Thus, investigation is necessary to lay a foundation for the further understanding of the interactions among plant, autotoxins and microbes.

In this study, the 'autotoxic circle' of *R. glutinosa* was evaluated by seedling tests and verified by plantlet

tests. We then quantified five central focal phenolic acids (4-hydroxybenzoic acid, vanillic acid, syringic acid, 3,4-dihydroxybenzoic acid and vanillin) in allelopathic research and provided a more precise identification and confirmation of potential autotoxins to understand *R. glutinosa* autotoxicity. Twenty new compounds emerged in the ‘autotoxic circle’, four of which were chosen based on the availability of the chemicals, the specific dimensionality distribution in the soil and previous reports to track their autotoxicity. Finally, the bacterial and fungal community distributions in the root-zone were investigated by PCR-DGGE.

Materials and methods

Container-culture experiment

Soil from Jiaozuo City that had never been used for *R. glutinosa* planting was selected for this container-culture experiment. The *R. glutinosa* cultivar ‘Wen 85-5’ is widely planted in the main production region. It was used as the seed and cultivated in the centre of the pots (diameter 100 cm, depth 40 cm) based on the production regulation. The following root-zone soil samples were subsequently collected: close soil (CS), including the soil collected after the harvest roots were shaken and the soil brushed from the surface of the roots; intermediate soil (IS), including the soil within 10 to 20 cm of the centre of the pots and distant soil (DS), including the soil within 20 to 30 cm of the centre of the pots. The soil from the pots that was not cultivated with *R. glutinosa* was collected as a control sample (CK). The soil cultivated with or without *R. glutinosa* was watered and managed equally, and the surface soils (3 cm deep) were abandoned before sampling to exclude environmental influences. All of the above soil samples were air-dried, blended and sieved through a 2-mm mesh for subsequent analysis.

Assessment of ‘autotoxic circle’

To define the *R. glutinosa* ‘autotoxic circle’, a seedling test was conducted to estimate the autotoxic effect of the following root-zone soils: CS, IS, DS and CK were weighed (20 g) and transferred into Petri dishes in triplicate, and 10 mL of distilled water was sprayed every 3 days after 20 *R. glutinosa* seeds were planted in the soil. In addition, cyclopentaneacetic acid, 3-oxo-

2-(2-pentenyl)-, methyl ester, [1R-[1a,2b(Z)]]- (compound 2), trans-2-hexadecenoic acid (compound 10), *tert*-hexadecanethiol (compound 14), palmitoleic acid (compound 19) and their mixtures, which were identified and selected from the GC-MS results, were dissolved in a small volume of ethanol and transferred onto the double-layered filter paper in the Petri dishes. In a draft chamber, the solvents evaporated for 1 h. The filter paper that contained these compounds was moistened with 5 mL distilled water, and the final concentrations of the compounds in water were 2, 5 and 10 µg/mL. Distilled water was used as a control, and 20 *R. glutinosa* seeds were placed on the filter paper. All dishes were maintained at 26 °C under fluorescent lights for 11 h (0800 hours–2000 hours), and the fluorescent light intensity was $4.68 \pm 0.14 \times 10^3$ lx. The radicle length was measured after incubation for 7 days. Additionally, a plantlet test was performed to verify the ‘autotoxic circle’ under aseptic conditions, in which the influence of polar and non-polar compounds within different root-zone soils on plantlets root growth was assessed as follows: Soil samples were weighed (200 g) and extracted using 400 mL HPLC-grade methanol and *n*-pentane for 24 h, separately. Then, the extracts were filtered and diluted with corresponding solvents to 400 mL. Eighty millilitres methanol extraction, as well as 80 mL *n*-pentane extraction were transferred into glass bottles and evaporated in a draft chamber for 8 h. Segment of stems with four leaves, including the apical meristem approximately 3 cm in length from the tissue culture plantlets of *R. glutinosa*, were used as explants and cultured in the bottles according to the same conditions of the seedling tests. The MS formulation was used as the nutrient medium, and six replications of each soil sample extract were performed. After 4 weeks, the roots of plantlets were washed and scanned using an Epson Expression 10000XL scanner (Epson Co., Ltd., Long Beach, CA, USA). Root length was determined by analysing the scanned images using WinRHIZO version Pro2007d software (Regent Instruments Inc., Quebec, Canada).

Quantitative analysis of phenolic acids in different root-zone soils

The five phenolic acids (4-hydroxybenzoic acid, vanillic acid, syringic acid, 3,4-dihydroxybenzoic acid and vanillin) were analysed by HPLC, and their concentrations were determined by the resulting peak areas combined

with standard curves. The standard curves were plotted based on single and mixed phenolic acids. The methods were as follows: standardized phenolic acids (Sigma-Aldrich Co., MO, USA) were weighed (0.025 g) and dissolved in 25 mL Milli-Q water as single standards, and then each single standard was combined and diluted to different gradients (0.5, 1, 5 and 10 µg/mL) for the mixed standards. The injection samples for the soil samples were obtained by the following steps: soil samples were weighed (5.0 g) and extracted using 100 mL HPLC-grade ethyl acetate (Fisher, NJ, USA), dried via rotary evaporators and redissolved in 5 mL HPLC-grade methanol (Fisher, NJ, USA). The HPLC system was composed of an LC-20AD liquid chromatography system, a SPD-20A ultraviolet detector, a SIL-20A autosampler, a CTO-10AS column oven and a CBM-20A communication module (Shimadzu, Kyoto, Japan). The chromatographic separation was performed on an Agilent XDB-C18 column (5 µm, 250 mm × 4.6 mm; Santa Clara, CA, USA) using methanol (solvent A; 28:72, v/v) and 2 % acetic acid (solvent B) as a mobile phase at a flow rate of 0.8 mL/min. During the experiment, the injection volume was 20 µL and the chromatogram was monitored at a wavelength of 280 nm. The column temperature remained at 30 °C. The samples and standard solutions were filtered through a 0.22-µm membrane before injection. The stability was tested, and the percent of recovery rates was measured (Zhang et al. 2015).

Identification of compounds in different root-zone soils

The polar and non-polar chemicals in the soil samples were extracted as follows: soil samples were weighed (50 g) and extracted using 200 mL HPLC-grade methanol and *n*-pentane, separately. Then, extracts were dried in rotary evaporators before redissolving in a 5 mL homologous extraction. Extracted samples were filtered through a 0.22-µm membrane for injection. A GC-MS analysis was performed with Agilent 7890A gas chromatography, which interfaced with an Agilent 7000 mass selective detector equipped with an electron impact ion (ionization energy 70 eV). A 1 µL extracted sample was injected via a 'splitless' mode. The full-scan monitoring mode (*m/z* 50–1500) was employed as the detection mode. The capillary column was DB-5 (30 m × 0.25 mm × 0.25 µm, Agilent, USA). The oven temperature was set to 50 °C for 2 min, followed by a gradient of 4 °C/min up to 250 °C and then held for

2 min. Helium was used as the carrier gas at 1 mL/min. The interface was 200 °C, and the ion source was 250 °C.

Analysis of microbial community in different root-zone soils

PCR-DGGE technology was employed to analyse the microbial community of the soil samples. The implementation method was as follows: soil samples were weighed (500 mg) to extract total genomic DNA in triplicate using a Fast DNA Spin kit for soil (Q-Biogene, USA) following the manufacturer's specifications. Bacterial 16S ribosomal DNA (rDNA) was amplified using the universal bacterial primer pair GC-338F and 518 R, and fungal 18S rDNA was amplified using GC-Fung and NS1. In a final volume of 50 µL, the PCR amplifications of the bacteria were performed, which included 100 ng soil DNA, 3.2 µL dNTP (2.5 mM), 0.4 µL rTaq (5 U/µL), 1 µL GC-338F (20 mM), 1 µL 518 R (20 mM), 5 µL 10× PCR buffer and an amount of ddH₂O. The 50 µL PCR amplifications for fungi consisted of 100 ng soil DNA, 4 µL dNTP (2.5 mM), 0.5 µL rTaq (5 U/µL), 0.5 µL GC-Fung (20 µM), 0.5 µL NS1 (20 µM), 5 µL 10× PCR buffer and an amount of ddH₂O. The thermal cycling for bacteria consisted of 30 cycles of pre-denaturation at 94 °C for 4 min, denaturation at 94 °C for 30 s, renaturation at 55 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 10 min. The thermal cycling for fungi proceeded as follows: 30 cycles of pre-denaturation at 94 °C (5 min), denaturation at 94 °C (1 min), renaturation at 50 °C (45 s), extension at 72 °C (1 min) and final extension at 72 °C for 10 min. The DGGE was performed for bacteria using 8 % (w/v) acrylamide gel with a 35–55 % denaturant gradient and run in 1× TAE (Tris-acetate-EDTA) buffer for 4 h at 60 °C and 150 V. In addition, a DGGE was performed for fungi using 7 % (w/v) acrylamide gel with 15–35 % denaturant gradient and run in 1× TAE buffer for 6 h at 60 °C and 150 V.

After electrophoresis, the gels were stained for 30 min with SyBR green I nucleic acid stain and photographed using a Bio-Rad trans-illuminator (Bio-Rad Laboratories, Segrate, Italy) under UV light. The visually prominent bands from the gels were excised using sterilized pipette tips and then recycled using Ploy-Gel DNA Extraction Kit. Two microlitres of buffer containing DNA were used as a template for PCR using

the same primer pair but without the GC-clamp. The PCR products were purified and cloned into *Escherichia coli* DH5 using the PMD 18-T plasmid vector system for bacteria and pEASY-T for fungi to sequence. Three clones per band were sequenced. The 16S rDNA and 18S rDNA sequences were compared against those available in the NCBI database.

Calculations and statistical analyses

An index of the inhibition rate (IR) was used to compare the radicle length and the plantlet root length of treatments with the controls.

$$\text{IR} = \frac{\text{control} - \text{treatment}}{\text{control}} \times 100 \%$$

where IR >0 indicates growth inhibition and IR <0 indicates growth promotion (Li et al. 2012).

The samples' peaks from GC-MS results were identified by their mass spectra combined with a Kovats index (KI), which was calculated by the injection of a solution containing a homologous series of normal alkanes (C₈-C₄₀ purchased from ANPEL Scientific Instrument Co., Ltd., Shanghai, China).

$$\text{KI} = 100n + 100(t_x - t_n) / (t_{n+1} - t_n)$$

where n is the carbon number of normal alkanes, t_x is the retention time of the sample peak, t_n is the retention time of the n -alkanes peak eluted immediately before the sample peak and t_{n+1} is the retention time of the n -alkanes peak eluted immediately after the sample peak. The chemicals in the results that showed the highest KI similarity with the KI* of compounds listed in the NIST library were considered reliable.

For analysis of the molecular community profiles, the Molecular Analyst Fingerprinting software (version 1.61, BioRad, Veenendaal, NL) was used. From the molecular (PCR-DGGE) community fingerprints analysed by the Molecular Analyst software, the Shannon-Weaver index (H') and the evenness index (E) were calculated according to the aforementioned method (Garbeva et al. 2008). The most similar sequences in NCBI blasting results were selected and listed as the identification results. The data of seedlings' radicle length, plantlets' root length, Shannon-Wiener diversity index (H') and evenness index were subject to an analysis of variance by SPSS software. Each value was expressed as the mean of three replicates \pm SE.

Results

Assessment and verification of 'autotoxic circle' of *R. glutinosa*

The radicles' length of the *R. glutinosa* seedlings grew in the close soil, the intermediate soil was significantly inhibited and the radicle length in the distant soil was not statistically different from that of the control soil (Figs. 1 and 2). The strongest inhibitory effect was observed firstly in the close soil (IR = 27.52 %) and secondly in the intermediate soil (IR = 12.30 %). These results indicated that the autotoxic effect of *R. glutinosa* was significant within 20 cm (close soil and intermediate soil) of the plant, and this effect lessened considerably at a distance beyond 20 cm (distant soil).

To exclude the influence of microbes on the assessment of the autotoxic circle, the polar and non-polar compounds within different root-zone soils were extracted and their effects on root growth of plantlets were evaluated through tissue culture. The plantlet test results were similar to the seedling tests: The strongest inhibitory effect was observed in the close soil (IR = 61.92 %) and then in the intermediate soil (IR = 25.65 %). The plantlets' root length which treated with extraction of distant soil showed no significant difference with that of the control soil (Fig. 3). This similar result indicated that the different compounds' constitution within different root-zone soils resulted in the formation of *R. glutinosa* 'autotoxic circle'. The diameter of this 'autotoxic circle' was 40 cm.

Concentration of five phenolic acids in the root-zone soils

HPLC results showed that 4-hydroxybenzoic acid, vanillic acid, syringic acid, 3,4-dihydroxybenzoic acid and vanillin were present in all of the treatment soils and the control soil. A comparison of the concentration of these five phenolic acids showed that they were relatively higher in the close soil and intermediate soil than in the distant soil and control soil, and the distant soil did not present significant differences from the control soil (Fig. 4). Furthermore, the concentrations of 4-hydroxybenzoic acid, vanillic acid and syringic acid in the intermediate soil and close soil were at the same level, whereas the concentrations of 3,4-dihydroxybenzoic acid and vanillin were significantly higher in the intermediate soil than in the close soil. This

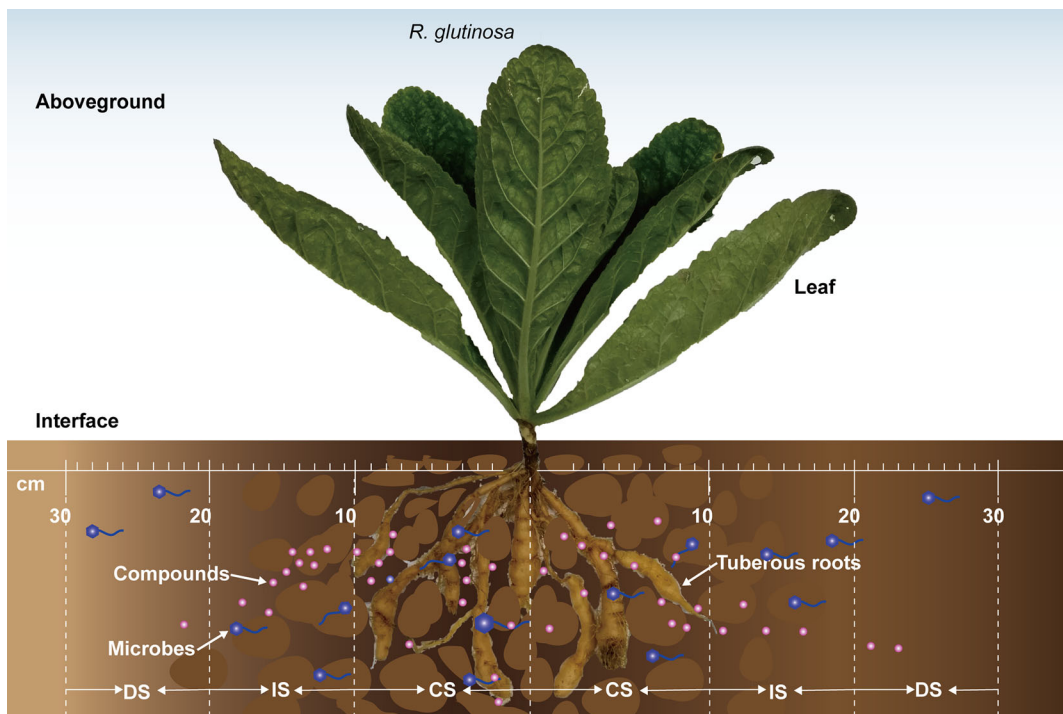


Fig. 1 Different root-zone soils of *R. glutinosa*. CS the close soil shaken and brushed from the *R. glutinosa* roots, IS the intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*

finding indicates that the concentration of phenolic acids in soil within 20 cm of *R. glutinosa* increased by the

cultivation of this plant, and the soil beyond 20 cm was unaffected.

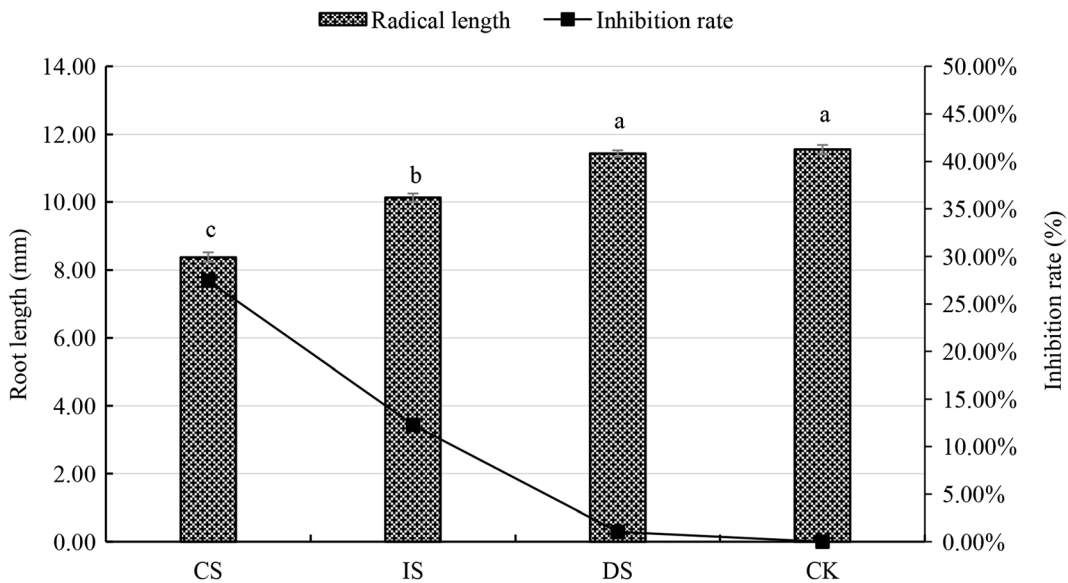


Fig. 2 Inhibition rate of different root-zone soils on radicle length of *R. glutinosa* seedlings. Different letters indicate significant differences among different radicle length at $P < 0.05$ according to Duncan's test. CS the close soil shaken and brushed from the *R.*

glutinosa roots, IS the intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*, CK the control soil that was not cultivated with *R. glutinosa*

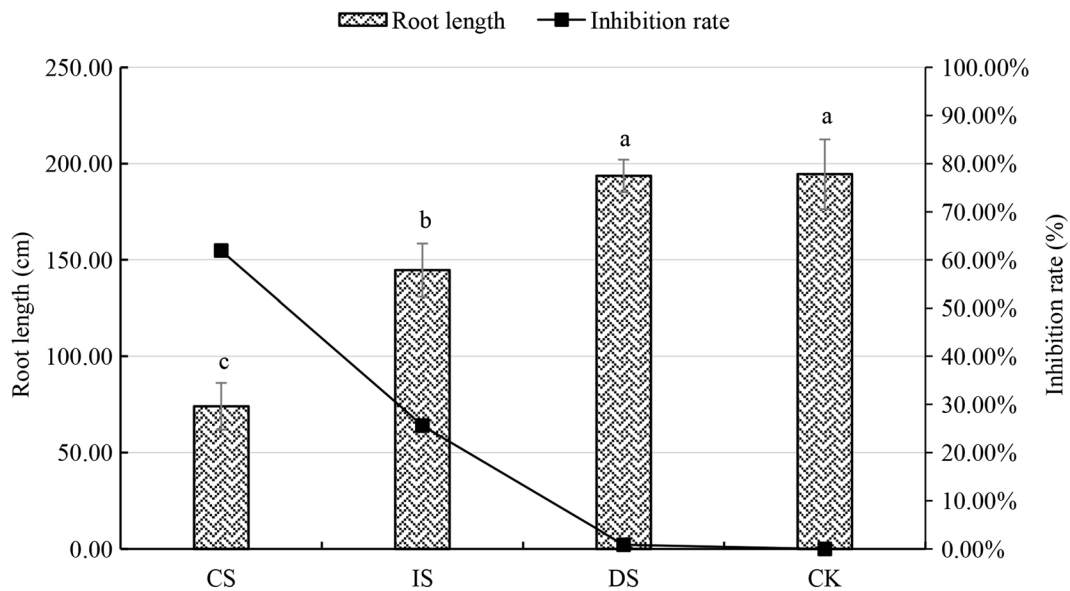


Fig. 3 Inhibition rate of extracts of different root-zone soils on root length of *R. glutinosa* tissue culture plantlets. Different letters indicate significant differences among different radicle length at $P < 0.05$ according to Duncan’s test. CS the close soil shaken and

brushed from the *R. glutinosa* roots, IS the intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*, CK the control soil that was never cultivated with *R. glutinosa*

Identification of autotoxins in the root-zone soils

The GC-MS spectrograms of the polar and non-polar fractions in the soil samples showed that the close soil containing the most peaks was followed by the intermediate soil. The spectrogram for the distant soil was highly similar to that of the control soil (Fig. 5). To

identify the specific chemicals originating from *R. glutinosa* cultivation, the control soil was used as the background, and 20 specific peaks (10 in methanol extraction and 10 in *n*-pentane extraction) were selected for identification by NIST database searching combined with KI screening. The molecular formula and KI of these chemicals were listed in Table 1. Notably, eight

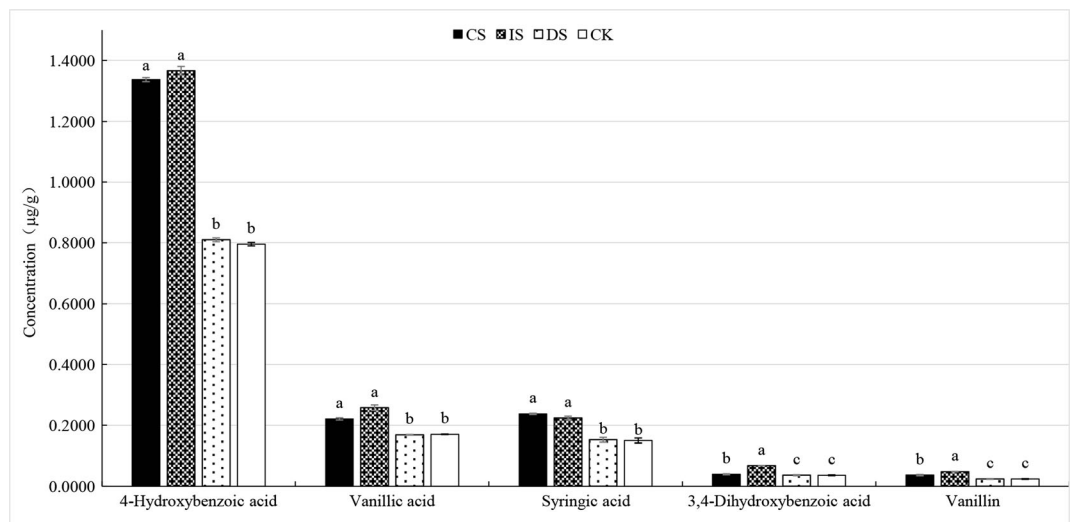


Fig. 4 Concentration of five phenolic acids in different root-zone soils. Different letters indicate significant differences among different concentrations at $P < 0.05$ according to Duncan’s test. CS the close soil shaken and brushed from the *R. glutinosa* roots, IS

the intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*, CK the control soil that was never cultivated with *R. glutinosa*

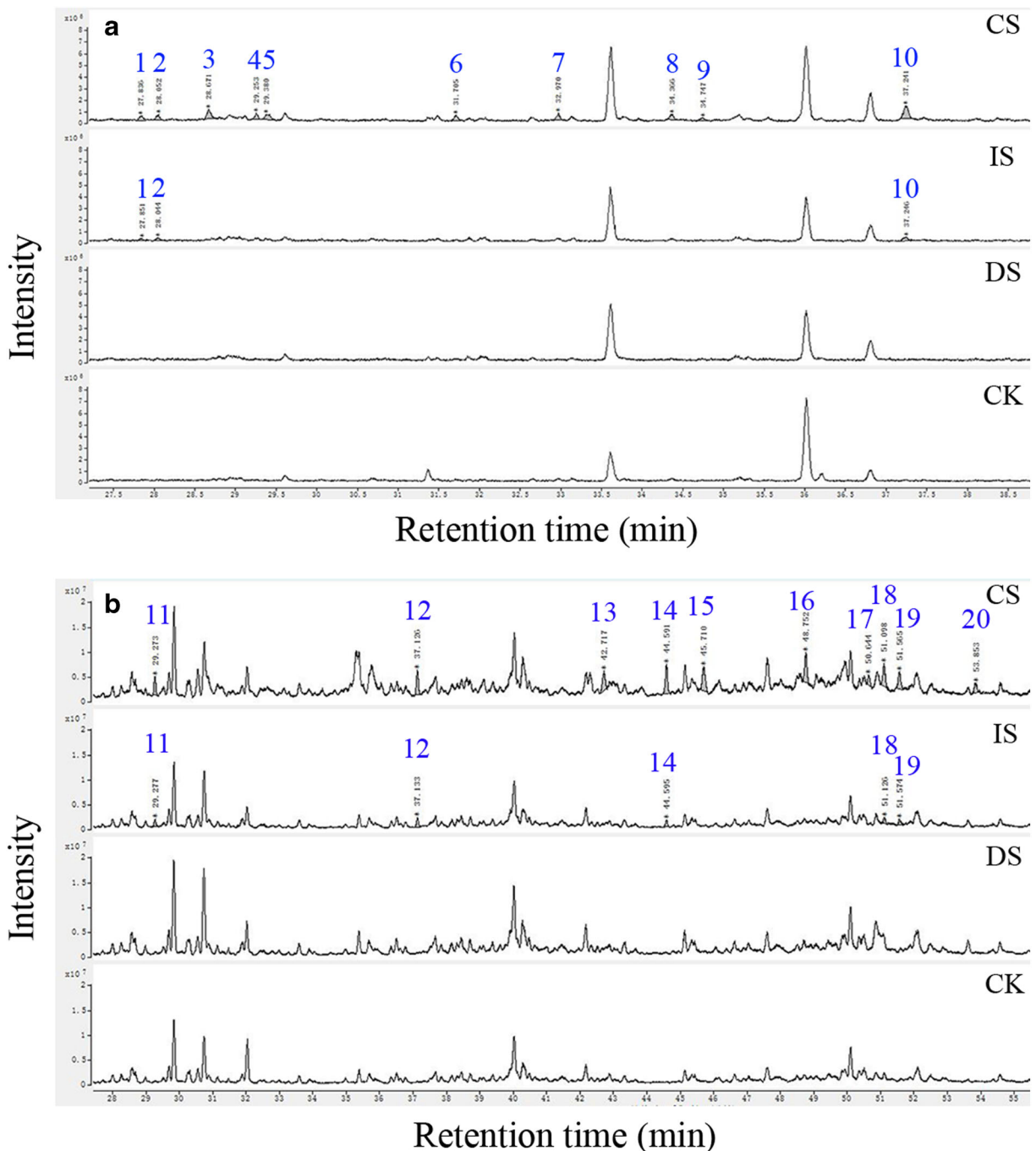


Fig. 5 GC-MS spectra of compounds in methanol (a) and *n*-pentane (b) extracts of different *R. glutinosa* root-zone soils. Same peak numbers indicate same compounds. CS the close soil that was shaken and brushed from the *R. glutinosa* roots, IS the

intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*, CK the control soil that was never cultivated with *R. glutinosa*

chemicals (compounds 1, 2, 10, 11, 12, 14, 18 and 20) present in the close soil were also specific to the intermediate soil. However, they were not detected

in the distant soil or control soil. Therefore, these eight compounds were derived from *R. glutinosa* cultivation.

Table 1 Identification of 20 compounds that specific to the close soil and intermediated soil

Peak number	Compound	MF	KI	KI*
1	2H-Oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-, [4S-(4R*,5E,10S*)]-	C ₁₀ H ₁₆ O ₃	1659	1656
2	Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-, methyl ester, [1R-[1a,2b(Z)]]-	C ₁₃ H ₂₀ O ₃	1667	1665
3	4-[2-(4-Methylphenyl) ethenyl] pyridin	C ₁₄ H ₁₃ N	1690	1681
4	1,3-Benzodioxole-5-carboxaldehyde, 4,7-dimethoxy-	C ₁₂ H ₁₂ O ₄	1710	1704
5	2,5-Diaminobenzoic acid	C ₁₃ H ₂₄ O ₂	1715	1773
6	8-Tetradecyn-1-ol acetate	C ₁₆ H ₂₈ O ₂	1794	1796
7	(7Z,10Z,13Z)-7,10,13-Hexadecatrienal	C ₁₆ H ₂₆ O	1833	1824
8	5-Dodecylbicyclo(2.2.1)-2-heptene	C ₁₉ H ₃₄	1875	1878
9	2-Hexadecenoic acid, methyl ester, (E)-	C ₁₇ H ₃₂ O ₂	1887	1886
10	<i>trans</i> -2-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	1962	1976
11	L-Arginine, methyl ester	C ₇ H ₁₆ N ₄ O ₂	1474	1464
12	4-Pentenoic acid, 4-(4-methylphenyl)-, ethyl ester	C ₁₄ H ₁₈ O ₂	1634	1638
13	Cyclododecanemethanol	C ₁₃ H ₂₆ O	1749	1743
14	<i>tert</i> -Hexadecanethiol	C ₁₀ H ₁₈ OS ₂	1787	1831
15	1-Hexadecanol	C ₁₆ H ₃₂ O ₂	1810	1864
16	Cyclohexane, 1,1'-(2-propyl-1,3-propanediyl) bis-	C ₁₈ H ₃₄	1874	1873
17	12-Dimethylamino-10-oxododecanoic acid	C ₁₄ H ₂₇ NO ₃	1914	1954
18	(9E)-9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	1924	1924
19	Palmitoleic acid	C ₁₆ H ₃₀ O ₂	1934	1930
20	Ethyl 9-hexadecenoate	C ₁₈ H ₃₄ O ₂	1983	1977

KI indicates the calculated Kovats index; KI* indicates the Kovats index of compounds listed in the National Institute of Standards and Technology (NIST) library. These 20 compounds were specific to the close and/or intermediate soil and were not detected in the distant soil and the soil cultivated without *R. glutinosa*

According to previous studies, some compounds detected in this work have been reported as allelochemicals in other plants (Cetin et al. 2010; Colvin and Gliessman 2011; Preston et al. 2004; Roy and Barik 2014; Tang and Gobler 2011). Based on these reports and the availability of the chemicals and the specific dimensionality distribution in the soil, four chemicals and their mixtures were selected and tested for their potential autotoxic effects on *R. glutinosa* radicle growth (Table 2). The results showed that these chemicals had different effects on seedling growth. Particularly, the seedling radicles were inhibited by 33.71–74.29 % when they were treated with the single compounds and mixtures at different concentrations. Additionally, hormesis, the phenomenon whereby a usually detrimental environmental agent (e.g., chemical substance, radiation) changes its role to provide beneficial effects when administered at low intensities or concentrations (Furst 1987), was

indicated by the radicle growth of *R. glutinosa* when the seeds were treated with compound 10 and compound 14 at a concentration of 2 µg/mL ($P < 0.05$). This stimulatory effect changed to an inhibitory effect at concentrations of 5 and 10 µg/mL. Moreover, seed germination was prevented (the radicle length was less than 1 mm) under treatment with the compound mixture and compound 2 at relatively higher concentrations (5 and 10 µg/mL compound mixture and 10 µg/mL compound 2). A comparison of effects on the IR among different compounds indicated that the compound mixture induced the strongest allelopathic effect on the seedling growth of *R. glutinosa*. The results demonstrated that these specific compounds extracted from the close and intermediate soils of *R. glutinosa* were potent inhibitors of seedling growth. There is a strong probability that these compounds belong to the autotoxins of *R. glutinosa*.

Table 2 Effect of the selected autotoxins on the radicle growth of *R. glutinosa*

	2 µg/mL		5 µg/mL		10 µg/mL		CK
	Radicle length (mm)	IR (%)	Radicle length (mm)	IR (%)	Radicle length (mm)	IR (%)	Radicle length (mm)
Compound 2	6.60±0.04b	46.12	3.54±0.03c	71.1	–	–	12.25±0.06a
Compound 10	13.45±0.01a	–9.8	6.00±0.03c	51.02	3.15±0.02d	74.29	12.25±0.06b
Compound 14	13.55±0.06a	–10.61	8.12±0.05c	33.71	5.24±0.04d	57.22	12.25±0.06b
Compound 19	7.64±0.04b	37.63	6.25±0.03c	48.98	3.52±0.03d	71.27	12.25±0.06a
Compound mixture	3.22±0.02b	73.71	–	–	–	–	12.25±0.06a

Data was the average values with standard errors of the replicates. *Different letters* indicate significant differences among different radicle length at $P < 0.05$ according to Duncan's test. Radicle lengths less than 1 mm were marked with '–'

PCR-DGGE analysis of the microbial community in different root-zone soils

The DGGE patterns showed that some microbes were only detected or showed relatively higher intensity in the corresponding soil range (Fig. 6).

These 23 soil-specific and individual density bands were excised and used as a template for PCR amplification. The sequence results of these PCR products were compared against those available in the NCBI database, and the closest relative species were listed (Tables 3 and 4).

As can be seen from the Tables 3 and 4, the sequences from these bands showed 94–100 % similarity to the closely related database sequences in GenBank. The close soil presented the most specific species (nine

bacterial species and four fungal species), followed by the intermediate soil (one bacterial species and three fungal species) and the distant soil (one bacterial species and one fungal species). Among these species, eight were uncultured species, and the others belong to different genera including *Chitinophaga*, *Hydrogenophaga*, *Bacillus*, *Pseudomonas*, *Ochrobactrum*, *Micrococcus*, *Ramicandelaber*, *Orbilia*, *Acremonium*, *Humicola*, *Heyderia*, *Lasiosphaeria*, *Dothidotthia* and *Coniosporium*. Additionally, the soil samples' Shannon-Wiener diversity index and evenness index of total bacteria, as well as total fungi, were calculated according to the 16S/18S rDNA PCR-DGGE banding patterns (Table 5). Overall, the microbial diversity and evenness index of the three soil types exhibited a descending order: close soil > intermediate

Fig. 6 DGGE profiles of the bacterial 16S rDNA (a) and fungal 18S rDNA (b) fragments. CS the close soil shaken and brushed from the *R. glutinosa* roots, IS the intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*, CK the control soil that was never cultivated with *R. glutinosa*. Three replicates for each soil

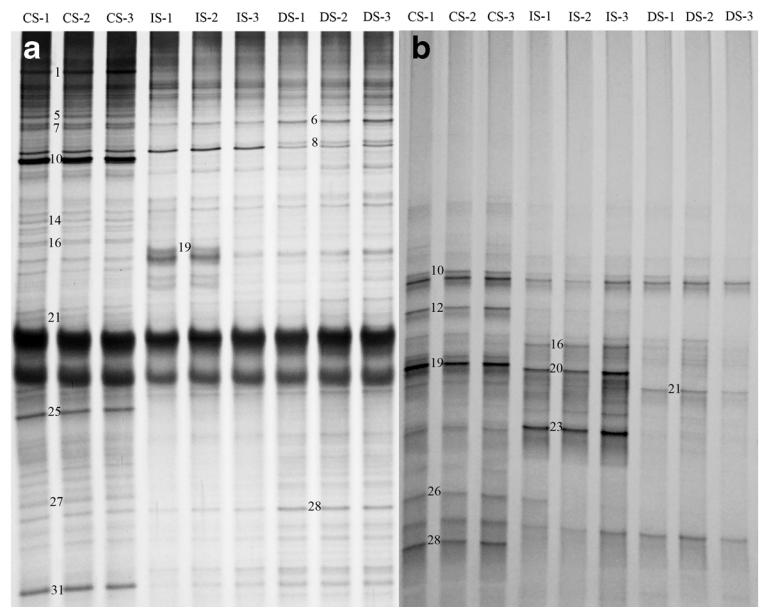


Table 3 Identification of specific microbial species in different *R. glutinosa* root-zone soils

Band name			Closest relative Species	Accession number	Similarity (%)	Phylogenic division
CS	IS	DS				
Band 1	–	–	Uncultured <i>Niastella</i> sp.	KC110902	94	<i>Niastella</i> ; environmental samples
Band 5	–	–	Bacterium FK5	EF462377	100	Bacteria
Band 6	Band 6	Band 6	Uncultured Actinobacterium	EU298580	100	Actinobacteria; environmental samples
Band 7	–	–	Uncultured Gemmatimonadetes bacterium	EU297301	100	Gemmatimonadetes; environmental samples
–	–	Band 8	Uncultured betaproteobacterium	JN409067	100	Betaproteobacteria; environmental samples
Band 10	–	–	<i>Chitinophaga</i> sp.	AB563185	98	Chitinophagaceae; <i>Chitinophaga</i>
Band 14	–	–	<i>Hydrogenophaga</i> sp.	KF013202	99	Comamonadaceae; <i>Hydrogenophaga</i>
Band 16	–	–	<i>Bacillus</i> sp.	KJ605142	100	Bacillaceae; <i>Bacillus</i>
–	Band 19	–	Uncultured Acidimicrobinea bacterium	JQ401244	99	Acidimicrobinea; environmental samples
Band 21	–	–	<i>Pseudomonas monteilii</i> strain	KJ806507	100	Pseudomonadaceae; <i>Pseudomonas</i>
Band 25	–	–	Uncultured bacterium	CU918269	100	Bacteria; environmental samples
Band 27	–	–	<i>Ochrobactrum</i> sp.	AB971024	100	Brucellaceae; <i>Ochrobactrum</i>
Band 28	Band 28	Band 28	Uncultured Bacteroidetes bacterium	HF564295	100	Bacteroidetes; environmental samples
Band 31	Band 31	Band 31	<i>Micrococcus</i> sp.	HG738944	100	Micrococcaceae; <i>Micrococcus</i>

CS the close soil shaken and brushed from the *R. glutinosa* roots, IS the intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*, ‘–’ the species were not found in the corresponding soil samples

soil > distant soil. In the close soil, the bacterial diversity index, the fungal diversity index and the fungal evenness index were significantly higher than that in the intermediate and distant soils, with values of 3.29 ± 0.032 , 2.97 ± 0.104 and 1.01 ± 0.040 , respectively. Significant differences in the bacterial evenness were not observed in these three soil samples. The findings presented here indicate that the microbial community changed due to *R. glutinosa* cultivation. The microbial constitution in the different *R. glutinosa* root-zone soils shifted dramatically, particularly within 20 cm of the plant that characterized by the appearance of various exclusive species. However, the microbes in the soil beyond this range were slightly affected.

Discussion

Various allelochemicals/autotoxins result in the plants' allelopathy/autotoxicity, and the autotoxicity is regarded as one of the important factors responsible for the plants' replant problems (Muller 1969; Singh et al. 2010; Zhu et

al. 2014). Our results demonstrated that the soil cultivated with *R. glutinosa* developed an autotoxicity effect on the growth of *R. glutinosa* seeds, and this effect decreased as the distance from the plant increased. Specifically, the autotoxic effect faded away at a distance beyond 20 cm. Moreover, the assessment of autotoxic effect under aseptic conditions showed similar results with soil culture conditions, implying that an 'autotoxic circle' was formed after *R. glutinosa* cultivation. This autotoxic range was induced by the compounds that appeared after *R. glutinosa* cultivation.

To further understand the autotoxins that induced *R. glutinosa* autotoxicity, phenolic acids, which were reported as the allelochemicals of many plants (Bais et al. 2006; Li et al. 2010), within the 'autotoxic circle' and beyond were analysed. The HPLC results showed that the concentrations of five phenolic acids within the 'autotoxic circle' were significantly higher compared with their concentrations in the soil beyond, implying that *R. glutinosa* cultivation increased the concentration of phenolic acids. According to the previous reports of *R.*

Table 4 Identification of specific fungal species in different *R. glutinosa* root-zone soils

Band name			Closest relative species	Accession number	Similarity (%)	Phylogenic division
CS	IS	DS				
Band 10	Band 10	Band 10	<i>Ramicandelaber longisporus</i>	KC297616	100	Kickxellaceae; <i>Ramicandelaber</i>
Band 12	–	–	<i>Arthrobotryis oligospora</i>	JQ809337	99	Orbiliaceae; <i>Orbilina</i>
–	Band 16	–	<i>Acremonium sclerotigenum</i>	HQ232209	100	Hypocreales incertae sedis; <i>Acremonium</i>
Band 19	–	–	<i>Humicola</i> sp.	DQ237874	99	Chaetomiaceae; <i>Humicola</i>
–	Band 20	–	<i>Heyderia abietis</i>	AY789288	99	Helotiaceae; <i>Heyderia</i>
Band 21	Band 21	Band 21	<i>Lasiosphaeria hispida</i>	JN938599	100	Lasiosphaeriaceae; <i>Lasiosphaeria</i>
–	Band 23	–	<i>Dothidothia aspera</i>	EU673228	100	Dothidothiaceae; <i>Dothidothia</i>
Band 26	–	–	Uncultured Cystofilobasidiales (aff. <i>Guehomyces</i>)	HQ326099	100	Cystofilobasidiales; environmental samples
Band 28	–	–	<i>Coniosporium</i> sp.	Y11712	97	Herpotrichiellaceae; <i>Coniosporium</i>

CS the close soil that was shaken and brushed from the *R. glutinosa* roots, IS the intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*, ‘–’ the species were not found in the corresponding soil samples

glutinosa, the allelopathy intensity of root exudates of different *R. glutinosa* growth stages showed positive correlation with its syringic acid concentration (Zhang et al. 2015). Seven phenolic acids were detected in the acetate-soluble extracts of *R. glutinosa* fibrous roots, and their bioassay showed a significant suppressive function on the seedling growth (Li et al. 2012). Similar autotoxic effects of phenolic acids were also reported in other plants. For instance, nine phenolic compounds (*p*-hydroxybenzoic acid, vanillin, syringic acid, vanillic acid, coumaric acid, ferulic acid, cinnamic acid, salicylic acid, benzoic acid) were detected in the

soil of commercially cultivated American ginseng, and all these compounds inhibited the radicle and shoot growth of itself (Bi et al. 2010). The autotoxic effect of ferulic acid and *p*-coumaric acid on cucumber and soybean was also reported (Politycka and Mielcarz 2007; Suzuki et al. 2008). Based on our results and these researches of other plants, these five phenolic acids are components of the autotoxins found in *R. glutinosa*. Although this evidences was powerful, it raised more questions than answer.

A noticeable phenomenon is that some preceding crops of *R. glutinosa* (such as wheat, maize) have been reported to release various phenolic acids, but they did not induced replant problems in *R. glutinosa* (Jia et al. 2014; Machinet et al. 2011; Maksimovic et al. 2008; Pham et al. 2011). However, the replant problems occurred when *R. glutinosa* was cultivated as a preceding crop of *R. glutinosa*. Why is it that the phenolic acids released by Gramineae crops did not induce the *R. glutinosa* replant problems? Based on the studies of other plants, the synergistic effect of allelochemicals drew our attention. Research on canola suggested that the synergistic effect of sinapyl alcohol, *p*-hydroxybenzoic and 3,5,6,7,8-pentahydroxy flavones played a role for canola allelopathy against annual ryegrass (Asaduzzaman et al. 2015). Research on boneseed's allelopathy indicated that the mixture of ferulic acid, catechin, phloridzin and *p*-coumaric acid showed a stronger inhibiting effect on *Isotoma axillaris* compared to individual phenolics (Al Harun et al. 2015). The combined allelopathic effect of three allelochemicals of *Chara vulgaris* also expressed

Table 5 Shannon-Wiener diversity and evenness indices based on the 16S rDNA/18S rDNA PCR-DGGE patterns

	Samples	Shannon-Wiener diversity index (<i>H'</i>)	Evenness (<i>E</i>)
Total bacteria	CS	3.29 ± 0.032a	0.98 ± 0.006a
	IS	2.97 ± 0.104b	0.97 ± 0.006a
	DS	2.94 ± 0.015b	0.96 ± 0.006a
Total fungi	CS	2.46 ± 0.035a	1.01 ± 0.040a
	IS	1.91 ± 0.058b	0.86 ± 0.025b
	DS	0.72 ± 0.021c	0.43 ± 0.015c

Data is the average values with standard errors of the replicates. Different letters indicate significant differences among different index at $P < 0.05$ according to Duncan's test. CS the close soil shaken and brushed from the *R. glutinosa* roots, IS the intermediate soil within 10 to 20 cm from the *R. glutinosa*, DS the distant soil within 20 to 30 cm from the *R. glutinosa*

synergistic inhibitory effects on the growth of *Microcystis aeruginosa* (Zhang et al. 2009). Thus, we hypothesized that there are some other *R. glutinosa* allelochemicals/autotoxins besides phenolic acids, and their synergistic effect exerts a stronger autotoxicity. Our GC-MS and bioassay results provided powerful evidence for this hypothesis. The GC-MS results showed that the variety of compounds decreased as the distance increased from the close soil, to the intermediate soil, to the distant soil; the compounds' constitution in the distant soil was very similar to the control soil that was not planted with *R. glutinosa*. Moreover, some compounds were only detected in the close and intermediate soil, implying these compounds were specific to the soil within the 'autotoxic circle' and emerged after the *R. glutinosa* cultivation. To track their potential autotoxic effect, four of the compounds were chosen for bioassay. The bioassay illustrated the following results: the trans-2-hexadecenoic acid (compound 10) and the tert-hexadecanethiol (compound 14) promoted the radicle growth with relative lower concentration, while inhibiting the radicle growth with higher concentrations, and the methyl jasmonate (compound 2) and the palmitoleic acid (compound 19) inhibited the radicle growth with even low concentrations. We suggest that they are the unreported members of *R. glutinosa* autotoxins. Additionally, a synergistic effect, which characterized by the inhibitory effect of the mixtures of these four compounds were much stronger than each single compound, was found. Similar results indicated that methyl jasmonate released from sagebrush had an allelopathic effect on *Nicotiana attenuata* when combined with other compounds (Preston et al. 2002). Therefore, there is a high probability that *R. glutinosa* autotoxicity is expressed by the synergistic effect of various autotoxins, including phenolic acids and other autotoxins (such as methyl jasmonate and other compounds listed in our result). The phenomenon of why the problems associated with replanted *R. glutinosa* do not occur after the cultivation of Gramineae crops may be partly explained by the absence of some other important autotoxins. Further studies are necessary to determine the synergistic effect of various autotoxins and to gain a more in-depth understanding of *R. glutinosa* autotoxicity. The identification of more potential autotoxins by combining GC-MS with other instruments (like LC-TOF-MS, LC-NMR) would be useful.

When tracking the potential functions of the identified microbes, we found that most of them were still unknown. However, some species belong to the same genus as our

identified species were confirmed to have positive effect on plant growth. For instance, some species of *Bacillus*, *Pseudomonas*, *Acremonium* and *Humicola* could promote plant growth due to their antagonistic activity, the production of IAA or specific substance to activate resistance for control of diseases (Dharni et al. 2014; Hassan et al. 2015; Khabbaz et al. 2015; Wenhsung 2009; Yang et al. 2014). On the other side, some species of these genera showed negative effect on plant growth due to their broad-spectrum pathogenic activity (Elbanna et al. 2014; Furuya et al. 2005; Iakovleva et al. 2013; Racedo et al. 2013; Zhang et al. 2014). Additionally, the report of other plant suggested that the harmful microbes were increased with the decrease of beneficial microbes under monocultivated conditions, and the soil ecological function was affected (Lin et al. 2015). Our previous report confirmed that continuous cropping of *R. glutinosa* decreased the bacteria species and simplified the bacterial community structure (Zhang et al. 2010). Thus, the waxing and waning of specific microbes may be also one important reason for the *R. glutinosa* replanted problem. More importantly, two identified species (*Arthrotrichy oligospora*, *Acremonium sclerotigenum*) may play an important role in the micro-ecological of *R. glutinosa* rhizosphere according to previous reports. It was confirmed that *A. oligospora* not only increased the plant growth and enhanced nutritional value of tomato but also functioned in controlling nematodes (Niu and Zhang 2011; Singh et al. 2013; Yang et al. 2011a). *A. sclerotigenum* was confirmed to be the pathogen of brown spot disease in apple (Li et al. 2014). Further studies on whether and how these two species shift under consecutive mono-cultivated conditions would be meaningful.

Furthermore, the newly research results of our team indicated that phenolic acid mixture could promote mycelial growth, sporulation and toxin production of *Fusarium oxysporum* while inhibiting growth of the *Pseudomonas* sp. W₁₂ (Wu et al. 2015). Research has confirmed that *F. oxysporum* is the pathogenic pathogens of *R. glutinosa* disease (Li et al. 2013) and that most strains of *Pseudomonas* showed antagonistic activity against *F. oxysporum* (Borah et al. 2015; Chin-A-Woeng et al. 2000; Srinivasan et al. 2009). In this work, we found that some congeneric species showed a close interaction with allelochemicals. For instance, *Pseudomonas* catalysed (-)-catechin conversion into 3,4-dihydroxybenzoic acid, and 3,4-dihydroxybenzoic acid showed a higher phytotoxicity than (-)-catechin

(Masuda et al. 2007); *Bacillus aquimaris* involved in the degradation of allelopathic *m*-tyrosine (Khan et al. 2013); *F. oxysporum*, a replant disease pathogen in *P. heterophylla* rhizospheric soil, increased sharply under a consecutive-replant system, and some phenolic acids promoted the growth of *F. oxysporum* f.sp. (Zhao et al. 2015). In production of *R. glutinosa*, 15 to 20 years of crop rotation is taken for its replanting (Yang et al. 2011b). In other words, the auto-inhibitory effect of *R. glutinosa* could last for a long time, and the farmland spends at least 15 years on itself-rehabilitation. During the process of formation and rehabilitation of the replant problems, some specific microbes may play an important role to catalyse, to transform and to degrade the autotoxins. Thus, more studies should be conducted not only on the various soil microbes, the autotoxins and the plants' abnormal metabolism but also on their interactions and functions in mediating plants' replant problems.

Conclusions

To summarize, the 'autotoxic circle' of *R. glutinosa* was firstly assessed in this work. Moderate yield practices have surpassed high yield practices to guarantee the concentration of active ingredients in traditional herb production. Defining the range of *R. glutinosa*'s autotoxic effect provided a refined map by which planting density can be optimized and the corresponding standards can be set in GAP and GMP. Moreover, we performed a more precise identification of several potential autotoxins of *R. glutinosa* based on the 'autotoxic circle'. Our results indicated that there are other *R. glutinosa* autotoxins in addition to phenolic acids. The synergistic effect of various potential autotoxins may contribute to the manifestation of their autotoxic activity. Furthermore, we found that many specific species emerged in the root-zone soils after *R. glutinosa* planting. One of these species is a potential pathogen, and another is a potential plant growth promoting fungus. This plant beneficial fungus may be utilized in the alleviation of *R. glutinosa* replanted problems. However, in addition to harness the power of this species, we need to understand how and why it matters with *R. glutinosa*. Future research should investigate the identification of other potential autotoxins through the means of multiple technologies and verify the function of

specific microbes in the rhizosphere ecosystem.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Al Harun MAY, Johnson J, Uddin MN, Robinson RW (2015) Identification and phytotoxicity assessment of phenolic compounds in *Chrysanthemoides monilifera* subsp *monilifera* (boneseed). *Plos One* 10:e0139992. doi:10.1371/journal.pone.0139992
- Asaduzzaman M, Pratley JE, An M, Luckett DJ, Lemerle D (2015) Metabolomics differentiation of canola genotypes: toward an understanding of canola allelochemicals. *Front Plant Sci* 5: 765. doi:10.3389/Fpls.2014.00765
- Bais HP, Weir TL LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* 57:233–266
- Bertin C, Yang XH, Weston LA (2003) The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil* 256:67–83. doi:10.1023/A:1026290508166
- Bi XB, Yang JX, Gao WW (2010) Autotoxicity of phenolic compounds from the soil of American ginseng (*Panax quinquefolium* L. Allelopath J 25:115–121
- Borah SN, Goswami D, Lahkar J, Sarma HK, Khan MR, Deka S (2015) Rhamnolipid produced by *Pseudomonas aeruginosa* SS14 causes complete suppression of wilt by *Fusarium oxysporum* f.sp. *pisi* in *Pisum sativum*. *Biocontrol* 60:375–385. doi:10.1007/s10526-014-9645-0
- Cetin B et al (2010) Antimicrobial activities of essential oil and hexane extract of Florence fennel *Foeniculum vulgare* var. *Azoricum* (Mill.) Thell. against foodborne microorganisms. *J Med Food* 13:196–204. doi:10.1089/jmf.2008.0327
- Chin-A-Woeng TF, Bloemberg GV, Mulders IH, Dekkers LC, Lugtenberg BJ (2000) Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. *Mol Plant-Microbe Interact* : MPMI 13: 1340–1345. doi:10.1094/mpmi.2000.13.12.1340
- Colvin WI III, Gliessman SR (2011) Effects of fennel (*Foeniculum vulgare* L.) interference on germination of introduced and native plant species. *Allelopath J* 28:41–51
- Dharni S, Srivastava AK, Samad A, Patra DD (2014) Impact of plant growth promoting *Pseudomonas monteilii* PsF84 and *Pseudomonas plecoglossicida* PsF610 on metal uptake and production of secondary metabolite (monoterpenes) by rose-scented geranium (*Pelargonium graveolens* cv. *bourbon*) grown on tannery sludge amended soil. *Chemosphere* 117: 433–439. doi:10.1016/j.chemosphere.2014.08.001

- Dixon RA, Strack D (2003) Phytochemistry meets genome analysis, and beyond. *Phytochemistry* 62:815–816. doi:[10.1016/S0031-9422\(02\)00712-4](https://doi.org/10.1016/S0031-9422(02)00712-4)
- Elbanna K, Elnaggar S, Bakeer A (2014) Characterization of *Bacillus altitudinis* as a new causative agent of bacterial soft rot. *J Phytopathol* 162:712–722. doi:[10.1111/jph.12250](https://doi.org/10.1111/jph.12250)
- Furst A (1987) Hormetic effects in pharmacology: pharmacological inversions as prototypes for hormesis. *Health Phys* 52:527–530. doi:[10.1097/00004032-198705000-00001](https://doi.org/10.1097/00004032-198705000-00001)
- Furuya H, Tubaki K, Matsumoto T, Fuji SI, Naito H (2005) Deleterious effects of fungi isolated from paddy soils on seminal root of rice. *J Gen Plant Pathol* 71:333–339
- Garbeva P, van Elsas JD, van Veen JA (2008) Rhizosphere microbial community and its response to plant species and soil history. *Plant Soil* 302:19–32. doi:[10.1007/s11104-007-9432-0](https://doi.org/10.1007/s11104-007-9432-0)
- Guo HR, Cui HY, Jin H, Yan ZQ, Ding L, Qin B (2015) Potential allelochemicals in root zone soils of *Stellera chamaejasme* L. and variations at different geographical growing sites. *Plant Growth Regul* 77:335–342. doi:[10.1007/s10725-015-0068-4](https://doi.org/10.1007/s10725-015-0068-4)
- Hassan MN, Namood-e-Sahar, Shah SZU, Afghan S, Hafeez FY (2015) Suppression of red rot disease by *Bacillus* sp based biopesticide formulated in non-sterilized sugarcane filter cake. *Biocontrol* 60:691–702. doi:[10.1007/s10526-015-9673-4](https://doi.org/10.1007/s10526-015-9673-4)
- Iakovleva LM, Makhinia LV, Shcherbina TN, Ogorodnik LE (2013) *Micrococcus* sp.—the pathogen of leaf necrosis of horse-chestnuts (*Aesculus* L.) in Kiev. *Mikrobiol Z (Kiev Ukraine : 1993)* 75:62–67
- Jia X, Wang WK, Chen ZH, He YH, Liu JX (2014) Concentrations of secondary metabolites in tissues and root exudates of wheat seedlings changed under elevated atmospheric CO₂ and cadmium-contaminated soils. *Environ Exp Bot* 107:134–143. doi:[10.1016/j.envexpbot.2014.06.005](https://doi.org/10.1016/j.envexpbot.2014.06.005)
- Kato-Noguchi H, Thi HL, Teruya T, Suenaga K (2011) Two potent allelopathic substances in cucumber plants. *Sci Hortic* 129:894–897. doi:[10.1016/j.scienta.2011.04.031](https://doi.org/10.1016/j.scienta.2011.04.031)
- Kato-Noguchi H, Thi HL, Sasaki H, Suenaga K (2012) A potent allelopathic substance in cucumber plants and allelopathy of cucumber. *Acta Physiol Plant* 34:2045–2049. doi:[10.1007/s11738-012-0997-8](https://doi.org/10.1007/s11738-012-0997-8)
- Khabbaz SE, Zhang L, Caceres LA, Sumarah M, Wang A, Abbasi PA (2015) Characterisation of antagonistic *Bacillus* and *Pseudomonas* strains for biocontrol potential and suppression of damping-off and root rot diseases. *Ann Appl Biol* 166:456–471. doi:[10.1111/aab.12196](https://doi.org/10.1111/aab.12196)
- Khan F, Kumari M, Cameotra SS (2013) Biodegradation of the allelopathic chemical m-tyrosine by *Bacillus aquimaris* SSC5 involves the homogentisate central pathway (Retracted article. See vol. 9, e102854, 2014). *Plos One* 8:e75928. doi:[10.1371/journal.pone.0075928](https://doi.org/10.1371/journal.pone.0075928)
- Li ZH, Wang QA, Ruan XA, Pan CD, Jiang DA (2010) Phenolics and plant allelopathy. *Molecules* 15:8933–8952. doi:[10.3390/molecules15128933](https://doi.org/10.3390/molecules15128933)
- Li ZF, Yang YQ, Xie DF, Zhu LF, Zhang ZG, Lin WX (2012) Identification of autotoxic compounds in fibrous roots of *Rehmannia* (*Rehmannia glutinosa* Libosch.). *Plos One* 7:e28806. doi:[10.1371/journal.pone.0028806](https://doi.org/10.1371/journal.pone.0028806)
- Li ZF et al (2013) Isolation of highly pathogenic pathogens and identification of formae speciales of *Rehmannia glutinosa* L. Chinese. *J Eco-Agric* 21:1426–1433. doi:[10.3724/SP.J.1011.2013.30313](https://doi.org/10.3724/SP.J.1011.2013.30313)
- Li BH, Wang CC, Dong XL, Zhang ZF, Wang CX (2014) *Acremonium* brown spot, a new disease caused by *Acremonium sclerotigenum* on bagged apple fruit in China. *Plant Dis* 98:1012–1012. doi:[10.1094/Pdis-02-14-0113-Pdn](https://doi.org/10.1094/Pdis-02-14-0113-Pdn)
- Lin S, Dai LQ, Chen T, Li ZF, Zhang ZY, Lin WX (2015) Screening and identification of harmful and beneficial microorganisms associated with replanting disease in rhizosphere soil of *Pseudostellariae heterophylla*. *Int J Agric Biol* 17:458–466. doi:[10.17957/Ijab/17.3.14.224](https://doi.org/10.17957/Ijab/17.3.14.224)
- Ma RJ, Wang NL, Zhu H, Guo SJ, Chen DS (2009) Isolation and identification of allelochemicals from invasive plant *Ipomoea cairica*. *Allelopath J* 24:77–84
- Machinet GE, Bertrand I, Barriere Y, Chabbert B, Recous S (2011) Impact of plant cell wall network on biodegradation in soil: role of lignin composition and phenolic acids in roots from 16 maize genotypes. *Soil Biol Biochem* 43:1544–1552. doi:[10.1016/j.soilbio.2011.04.002](https://doi.org/10.1016/j.soilbio.2011.04.002)
- Maksimovic JD, Maksimovic V, Zivanovic B, Sukalovic VHT, Vuletic M (2008) Peroxidase activity and phenolic compounds content in maize root and leaf apoplast, and their association with growth. *Plant Sci* 175:656–662. doi:[10.1016/j.plantsci.2008.06.015](https://doi.org/10.1016/j.plantsci.2008.06.015)
- Masuda M, Yamasaki Y, Ueno S, Inoue A (2007) Isolation of bisphenol A-tolerant/degrading *Pseudomonas monteilii* strain N-502. *Extremophiles* 11:355–362. doi:[10.1007/s00792-006-0047-9](https://doi.org/10.1007/s00792-006-0047-9)
- Muller CH (1969) Allelopathy as a factor in ecological process. *Plant Ecol* 18:348–357. doi:[10.1007/BF00332847](https://doi.org/10.1007/BF00332847)
- Muscolo A, Sidari M (2006) Seasonal fluctuations in soil phenolics of a coniferous forest: effects on seed germination of different coniferous species. *Plant Soil* 284:305–318. doi:[10.1007/s11104-006-0040-1](https://doi.org/10.1007/s11104-006-0040-1)
- Niu X-M, Zhang K-Q (2011) *Arthrobotrys oligospora*: a model organism for understanding the interaction between fungi and nematodes. *Mycology* 2:59–78
- Olofsdotter M, Rebulanan M, Madrid A, Wang DL, Navarez D, Olk DC (2002) Why phenolic acids are unlikely primary allelochemicals in rice. *J Chem Ecol* 28:229–242. doi:[10.1023/A:1013531306670](https://doi.org/10.1023/A:1013531306670)
- Pham VH, Hatcher DW, Barker W (2011) Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. *Food Chem* 126:1896–1901. doi:[10.1016/j.foodchem.2010.12.015](https://doi.org/10.1016/j.foodchem.2010.12.015)
- Politycka B, Mielcarz B (2007) Involvement of ethylene in growth inhibition of cucumber roots by ferulic and p-coumaric acids. *Allelopath J* 19:451–460
- Preston CA, Betts H, Baldwin IT (2002) Methyl jasmonate as an allelopathic agent: sagebrush inhibits germination of a neighboring tobacco, *Nicotiana attenuata*. *J Chem Ecol* 28:2343–2369. doi:[10.1023/A:1021065703276](https://doi.org/10.1023/A:1021065703276)
- Preston CA, Laue G, Baldwin IT (2004) Plant-plant signaling: application of trans- or cis-methyl jasmonate equivalent to sagebrush releases does not elicit direct defenses in native tobacco. *J Chem Ecol* 30:2193–2214. doi:[10.1023/B:JOEC.0000048783.64264.2a](https://doi.org/10.1023/B:JOEC.0000048783.64264.2a)
- Racedo J, Salazar SM, Castagnaro AP, Ricci JCD (2013) A strawberry disease caused by *Acremonium strictum*. *Eur J Plant Pathol* 137:649–654. doi:[10.1007/s10658-013-0279-3](https://doi.org/10.1007/s10658-013-0279-3)
- Roy N, Barik A (2014) Long-chain free fatty acids from sunflower (*Asteraceae*) leaves: allelochemicals for host location by the

- Arctiid moth, *Diacrisia casignetum* Kollar (Lepidoptera: Arctiidae). *J Kansas Entomol Soc* 87:22–36
- Seigler DS (1998) Plant secondary metabolism plant secondary metabolism 76-93
- Singh NB, Singh A, Singh D (2010) Autotoxicity of maize and its mitigation by plant growth promoting rhizobacterium *Paenibacillus polymyxa*. *Allelopath J* 25:195–204
- Singh UB et al (2013) *Arthrobotrys oligospora*-mediated biological control of diseases of tomato (*Lycopersicon esculentum* Mill.) caused by *Meloidogyne incognita* and *Rhizoctonia solani*. *J Appl Microbiol* 114:196–208. doi:10.1111/jam.12009
- Srinivasan K, Gilardi G, Garibaldi A, Gullino ML (2009) Efficacy of bacterial antagonists and different commercial products against *Fusarium wilt* on rocket. *Phytoparasitica* 37:179–188. doi:10.1007/s12600-009-0024-9
- Suzuki LS, Zonetti PC, Ferrarese MLL, Ferrarese O (2008) Effects of ferulic acid on growth and lignification of conventional and glyphosate-resistant soybean. *Allelopath J* 21:155–163
- Tang YZ, Gobler CJ (2011) The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy. *Harmful Algae* 10:480–488. doi:10.1016/j.hal.2011.03.003
- Wang MD, Chen HG, Liu XY, Gao YQ, Wu K, Jia XC (2010) Lauric acid and 2, 6-ditertbutyl phenol, two major allelochemicals from *Rehmannia glutinosa* inhibiting the germination of succeeding crop, *Sesamum indicum*. *Afr J Biotechnol* 9:8672–8678
- Wenhsung K (2009) Nature of slow and quick decline of macadamia trees. *Bot Stud* 50:1–10
- Weston LA, Ryan PR, Watt M (2012) Mechanisms for cellular transport and release of allelochemicals from plant roots into the rhizosphere. *J Exp Bot* 63:3445–3454. doi:10.1093/jxb/ers054
- Wu LK, Wang HB, Zhang ZX, Lin R, Zhang ZY, Lin WX (2011) Comparative metaproteomic analysis on consecutively *Rehmannia glutinosa*-monocultured rhizosphere soil. *Plos One* 6:e20611. doi:10.1371/journal.pone.0020611
- Wu LK et al (2015) Plant-microbe rhizosphere interactions mediated by *Rehmannia glutinosa* root exudates under consecutive monoculture. *Sci Report* 5:15871. doi:10.1038/Srep1587
- Yang YH, Chen XJ, Chen JY, Xu HX, Zhang ZY (2011a) Differential miRNA expression in *Rehmannia glutinosa* plants subjected to continuous cropping. *Bmc Plant Biol* 11:53. doi:10.1186/1471-2229-11-53
- Yang J et al (2011b) Genomic and proteomic analyses of the fungus *Arthrobotrys oligospora* provide insights into nematode-trap formation. *PLoS Pathog* 7:e1002179. doi:10.1371/journal.ppat.1002179
- Yang CH, Lin MJ, Su HJ, Ko WH (2014) Multiple resistance-activating substances produced by *Humicola phialophoroides* isolated from soil for control of *Phytophthora* blight of pepper. *Bot Stud* 55:1–10
- Zhang TT, He M, Wu AP, Nie LW (2009) Allelopathic effects of submerged macrophyte *Chara vulgaris* on toxic *Microcystis aeruginosa*. *Allelopath J* 23:391–401
- Zhang ZY, Chen H, Yang YH, Chen T, Lin RY, Chen XJ, Lin WX (2010) Effects of continuous cropping on bacterial community diversity in rhizosphere soil of *Rehmannia glutinosa*. *Chin J Appl Ecol* 21:2843–2848
- Zhang ZY, Lin WX, Yang YH, Chen H, Chen XJ (2011) Effects of consecutively monocultured *Rehmannia glutinosa* L. On diversity of fungal community in rhizospheric soil. *Agric Sci China* 10:1374–1384. doi:10.1016/S1671-2927(11)60130-2
- Zhang JB, An M, Wu HW, Liu DL, Stanton R (2012) Chemical composition of essential oils of four *Eucalyptus* species and their phytotoxicity on silverleaf nightshade (*Solanum elaeagnifolium* Cav.) in Australia. *Plant Growth Regul* 68:231–237. doi:10.1007/s10725-012-9711-5
- Zhang LX, Li SS, He T, Tan GJ (2014) Identification and pathogenicity of *Pseudomonas fluorescens* associated with canker disease of kiwifruit in Central China. *J Hortic Sci Biotechnol* 89:130–135
- Zhang B, Li XZ, Feng FJ, Gu L, Zhang JY, Zhang LJ, Zhang ZY (2015) Correlation of allelopathy of *Rehmannia glutinosa* root exudates and their phenolic acids contents. *J Chin Med Mater* 38:659–663. doi:10.13863/j.issn1001-4454.2015.04.002
- Zhao YP et al (2015) Interaction of *Pseudostellaria heterophylla* with *Fusarium oxysporum* f.sp *heterophylla* mediated by its root exudates in a consecutive monoculture system. *Sci Report* 5:8197. doi:10.1038/Srep08197
- Zhu XZ, Guo J, Shao H, Yang GQ (2014) Effects of allelochemicals from *Ageratina adenophora* (Spreng.) on its own autotoxicity. *Allelopath J* 34:253–264
- Zuo SP, Fang ZS, Yang SY, Kun W, Han YJ (2015) Effect of allelopathic potential from selected aquatic macrophytes on algal interaction in the polluted water. *Biochem Syst Ecol* 61:133–138. doi:10.1016/j.bse.2015.06.011