Effect of activated carbon on phenolic acids and microbes in the Rehmannia glutinosa succession cropping soils

Yupeng He[1, 2,a], Nianxi Zhao[3,b],Hongpeng Yang[2,c,] Jinlong Wang[2,d*,] Xidong Wu[2,e]

[1]College of Agriculture & Biotechnology, Zhejiang University, Hangzhou,China

[2]College of Agronomy & Resources and Environment, Tianjin Agriculture University, Tianjin,China

[3]College of Life Science, Nankai University, Tianjin, China

[a]hebobo329light@163.com, [b]zhaonianxi@nankai.edu.cn ,[c]yanghongpeng2003@163.com, [d]wangjinlong@tjau.edu.cn(*Corresponding author), [e]wuxidong@tjau.edu.cn

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Abstract. In this paper, different proportion of activated carbon was added to the Rehmannia glutinosa continuous cropping soils including first and second stubble to adjust the phenolic acids content and microbial biomass and improve the soil environment after continuous cropping. Results showed that vanillic acid, vanillin and ferulic acid in the continuous cropping soils were all somewhat decreased after a growth cycle, half a year. The ratio of fungi to bacteria was increased in the soil cropped Rehmannia glutinosa for one year, while decreased in the second stubble soil after addition of activated carbon. Taken together, the effect, 0.8 percent addition of activated carbon to the soil cropped Rehmannia glutinosa for two years, was the best.

Introduction

Rehmannia glutinosa L. is a perennial herbaceous species belonging to the *Scrophulariaceae* family, and is widely cultivated in Henan, Shanxi, Shandong, Anhui, and Jilin Province in China. The medicinal value of *Rehmannia glutinosa* was recorded and narrated in *Sheng Nong's herbal classic,* the earliest medicine monographs in China. Modern research showed that *Rehmannia glutinosa,* which belongs to commonly used Chinese medicinal herbs, has the functions of regulating blood sugar, protecting liver, hemostasis, diuresis, antiphlogosis and so on. It's root and processed products were often used medicinally [1]. It has been an accepted fact for hundreds of years that *Rehmannia glutinosa* can't be cultured without rotation. Easing the *Rehmannia glutinosa* continuous cropping obstacle has become a hot spot problem in the world because of the large demand and limited suitable planting regions.

Rhizosphere microecological imbalance may be the key factor in causing Rehmannia glutinosa continuous cropping obstacles, whichhas been proved by many researchers [2-4]. Meanwhile, some researchers found that phenolic acids secreted from *Rehmannia glutinosa* were the key causes of continuous cropping obstacles [5]. The addition of activated carbon to the soil could not only adsorb allelochemicals excreted from plant roots, remove adverse influences caused by allelochemicals [6, 7], but also increase the amount of microorganism in soils [8-10].

Therefore, activated carbon was added in the continuous cropping soils in this paper to try to adjust phenolic acids content and microbial biomass and improve continuous cropping soil environment.

Materials and Methods

Materials

The soils used in this experiment were all half silty loam, including ones cropped Rehmannia glutinosa for one and two years, and ones had never cropped rehmannia. The soils were all taken from Yuncheng, Shanxi Province in China. Activated carbon was bought from Hongyan Chemical Reagent Factory in Tianjin Province.

Experiment design

Double factors random block was used in this experiment. The first factor was soil, including ones cropped rehmannia for one and two years and ones had never cropped rehmannia. The second factor was activated carbon, including low addition amount (0.8%, weight ratio with soil, the same as follows) and high addition amount (1.6%). Each dosage of activated carbon mixed evenly with soils cropped rehmannia for one and two years. Soil never cropped rehmannia did not do any treatment. There was a group in each kind of soils treated as control and never mixed with additives.

Poured the mixed soil into pots (18×19cm) and put the fitted gauze bag at the bottom of the pots in case of earth leakage. The soil weight of each pot was 2.535kg contained exogenous additive. Water the soils regularly to keep the moist. All experiment was conducted in greenhouse of Naikai University from October 17, 2012 to April 28, 2013.

Determination method of phenolic acid

The content of phenolic acids in the soils were determined by HPLC[11, 12].

Soil extracting method [11, 12]

Soil samples (20g) were homogenized with 20ml NaOH (1mol/L) in centrifuge tubes (50ml) and stable at room temperature for all night after being sonicated in an ultrasonic bath for 30 min, and sonicated for another 30 min the next day before being centrifuged at 3000r/min for 20 min to get the supernatant. Concentrated hydrochloric acid (12mol/L) was added to the supernatant to decrease the PH to 2.5, then put the liquid at room temperature for 2 hours and centrifuged it to get rid of humic acid. The supernatant was extracted by same volume normal butanol 3 times. Extracts were combined and dried by rotary evaporator, and then dissolved by methanol to 2ml and filtered through organic membrane (0.45um), then a portion was injected onto the HPLC. The result was calculated by dry soil.

Instrument used in the experiment

The chromatographic system was Shimadzu LC-20AT HPLC which consisted of two LC-20AT infusion pumps, one SPD-M20A ultraviolet detector and a 20uL manual injector. Water Purification System was UPH-I-20L. Rotary evaporator was EYEL4OSB-2000 and ultrasonic bath was KH5200DB.

Chromatographic condition

The separation was achieved by using a 4.6×250mm, 5um, 100A C_{18} column (Venusll XBP, Tianjin, China) with a mobile phase consisting of NaAc (0.01mol/L), acetic acid, n-butyl alcohol and ammonium hydroxide, whose proportion was 100:0.15:2:0.05. The mobile phase was delivered at a flow rate of 0.7ml/min and detected at 277 nm. The injection volume was 5uL.

Standard substance and reagent

The standard substances of ferulic acid, p-hydroxybenzoic acid and vanillic acid were all bought from Shiji Aoke Company in Beijing. Vanilline standard was bought from Xiya Company in Sichuan. The methanol and n-butyl alcohol used in the mobile phase were all chromatogram class. Glacial acetic acid was analytical reagent made in China. All the mobile phase and samples were filtered through millipore filters (0.45um).

Determination method of microbial biomass

Plate count method was used to determine the biomass of soil microbe, among which bacteria was cultured in beef extract-peptone medium; fungus was cultured in Martin medium and actinomycetes was cultured in Improved Gaoshi No.1 medium. The biomass was counted and authenticated by conventional method [11].

Results and analysis

Chromatographic detection of phenolic acids

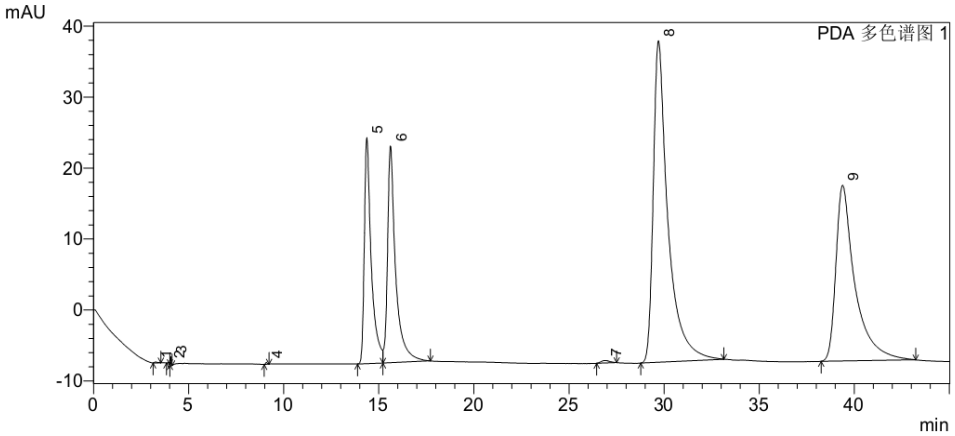

Figure 1. The chromatogram of mixed phenolic acids standard

(Peak 5, 6, 8 and 9 was p-hydroxybenzoic acid, vanillic acid, vanilline and ferulic acid, respectively)

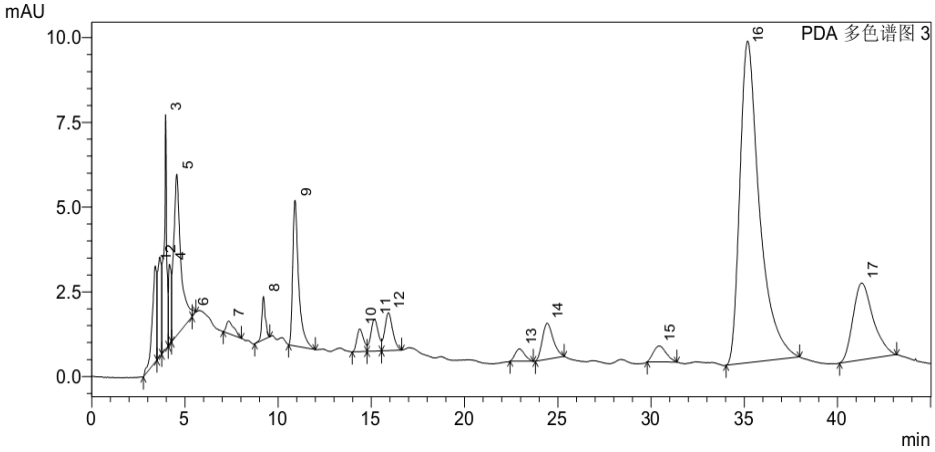

Figure 2. The chromatogram of phenolic acids of soil samples

(Peak 10, 12, 15 and 17 was p-hydroxybenzoic acid, vanillic acid, vanilline and ferulic acid, respectively)

From Figure 2, we could know chromatographic condition above could separate 4 kinds of phenolic acids absolutely, so this condition could be used to determine p-hydroxybenzoic acid, vanillic acid, vanilline and ferulic acid in soils.

Content variation of phenolic acids in soils

Chart 1. The content of phenolic acids in soils （ug/g）

		PHBA	Vanillic acid	Vanilline	Ferulic acid
Control （never crop）		0.13	0.17	0.00	0.33
Crop	Control	0.19	0.30	0.11	1.26
1	Low add	0.22	0.31	0.08	0.95
year	High add	0.17	0.18	0.00	0.33
Crop	Control	0.17	0.27	0.09	0.65
2	Low add	0.19	0.24	0.00	0.48
years	High add	0.21	0.23	0.00	0.73

The content of phenolic acids except p-hydroxybenzoic acid were all decreased with the increase of activated carbon addition, which proved the fact that activated carbon could adsorb phenolic acids [6, 7]. High addition (1.6%) of activated carbon could decrease the content of p-hydroxybenzoic acid partly in soils cropped *Rehmannia glutinosa* for one year. But in soils cropped *Rehmannia glutinosa* for two years, the content of p-hydroxybenzoic acid were increased with the increase of activated carbon addition.

Variation of soil microbe

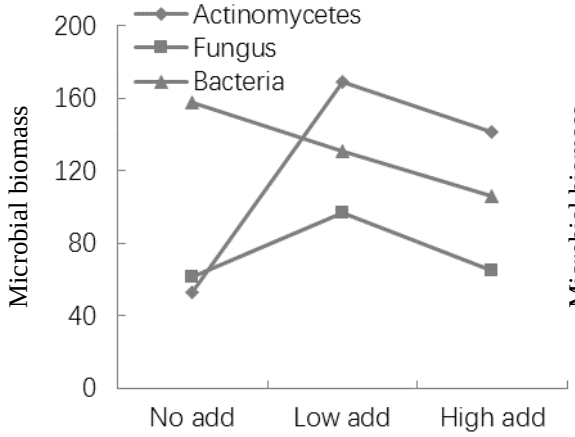

Figure 3. The biomass of 3 kinds of microbe in soils cropped rehmannia for one year

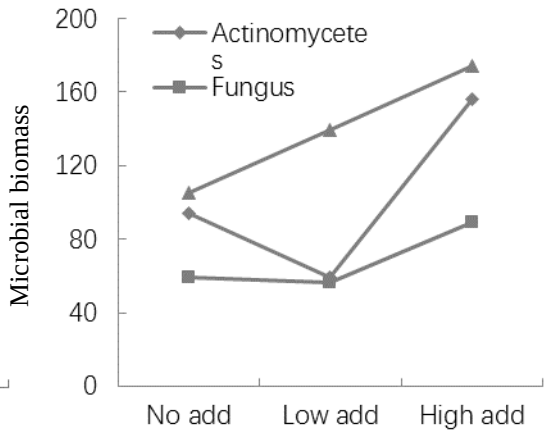

Figure 4. The biomass of 3 kinds of microbe in soils cropped rehmannia for two years

（PS: The unit of bacteria and actinomyces is 10^4 cuf.g^{-1} soil, fungus unit is 10^3 uf.g^{-1} soil）

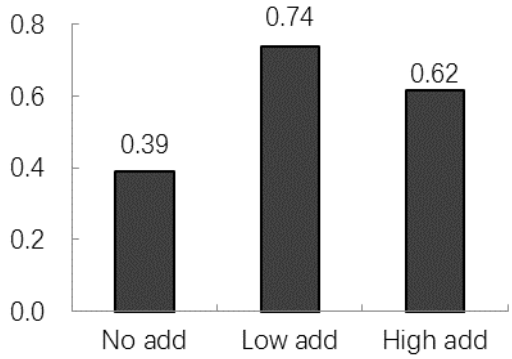

Figure 5. The ratio of fungi to bacteria in soils cropped rehmannia for one year

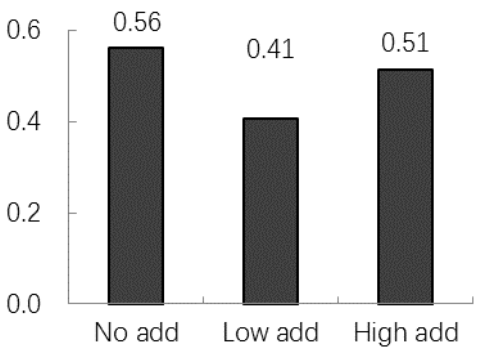

Figure 6. The ratio of fungi to bacteria in soils cropped rehmannia for two years

The amount of bacteria was decreased with the increase of activated carbon in soils cropped rehmannia for one year. But in soils cropped rehmannia for two years, the result was absolutely opposite. The amount of fungus and actinomyces were all increased firstly and then decreased with the increase of activated carbon in soils cropped rehmannia for one year, but the general trend was increasing. In soils cropped rehmannia for two years, high addition (1.6%) of activated carbon could increase the amount of fungus and actinomyces sharply, and low addition (0.8%) decreased the actinomyces amount partly.

The ratio of fungi to bacteria was increased firstly and then decreased with the increase of activated carbon in soils cropped rehmannia for one year, but the general trend was increasing. However, the result was completely opposite in soils cropped rehmannia for two years.

Discussion

From the result in this paper, we could get that the content of vanillic acid, vanilline and ferulic acid wereall decreased in the soils cropped rehmannia for one and two years after addition of activated

carbon, which proved the fact that phenolic acids in soils could be absorbed by activated carbon [6, 7]. At the same time, the ratio of fungi to bacteria was increased in the soils cropped rehmannia for one year after addition of activated carbon. However, the result was completely opposite in soils cropped rehmannia for two years.

Rehmannia is one of the plants that have the most serious continuous cropping obstacle. The recent researches of the reasons causes replant diseases were focus on microbial ecological environment in the rehmannia rhizosphere soils and allelochemicals secreted from rehmannia roots. Li's study [13] showed that rehmannia succession cropping would translate edaphon from bacteria domination to fungi domination and the increase of fungus amount would decrease soil fertility definitely.

Du's research [5] indicated that the key factor effected rehmannia successive cropping obstacle were allelochemicals, mostly phenolic acids, excreted from rehmannia roots. Among which ferulic acid played a major role in the inhibition of rehmannia continuous cropping [14]. Because ferulic acid is the prosthetic group of indoleacetic acid oxidase and can improve the IAAO activity and promote the reduction of indoleacetic acid [11,15], which is one of the major endogenous hormone help rehmannia roots grow [16]. So, the reduction of phenolic acids content and ratio of fungi to bacteria is helpful to successive cropping obstacle remission.

Study showed that activated carbon addition in soils can not only increase the soil microbial biomass [8-10] but also absorb phenolic acids excreted from rehmannia root [6, 7], then adjust the phenolic acids content and microorganism amount in continuous cropping soils to improve the continuous cropping soil environment. The result of this paper showed that low addition (0.8%) of activated carbon in soils cropped rehmannia for two years could decrease the ferulic acid content and ratio of fungi to bacteria half a year, a growth cycle, later, and the effect was the best. But the difference between soils cropped rehmannia for one and two years on microorganism has to be studied further.

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