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Inference of Allelopathy about Spartina Alterniflora to Scirpus Mariqueter by Effects of Activated Carbon on Soil

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Abstract

Spartina alterniflora Loisel is an invasive species in Jiuduansha Islands and threatens the survival of native species *Scirpus mariqueter*. In this study, activated carbon (AC) was applied to study the allelochemicals remained in the soil. Seed germination and seedling growth bioassays were used to test the allelopathic effect, and GC-MS was used to identify the allelochemicals. Our results showed: due to the invasion of *S. alterniflora*, germination of *S. mariqueter* seeds and the growth of seedlings were significantly inhibited. When AC was added into *S. mariqueter* soil, the germination had not been affected while the seedling growth was promoted significantly. When AC was added into the soil of *S. alterniflora*, both the germination and the seedling growth had an obvious improvement. All indicated that *S. alterniflora* soil contained allelochemicals which would be absorbed by AC. The identified allelochemicals were hexadecanoic acid, octadecanoic acid, dibutyl phthalate, (adipic acid, isohexyl methyl ester) and (adipic acid, di (oct-4-yl ester)).

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Keywords: Allelopathic effect- Soil- Activated carbon- Spartina alterniflora

Ⅰ. Introduction

Spartina alterniflora Loisel is a rhizomatous perennial, native to the Atlantic and Gulf coasts of North America. It was intentionally introduced to China in 1979 to accelerate sedimentation and land formation via so-called “ecological engineering”, and to the Yangtze Estuary in 1990’s [1,2,3, 4,5]. While the rapid growth of this species has greatly helped to stabilize tidal flats, it has the negative impacts of displacing native species [6]. It threatens the local biodiversity and the ecosystems, and especially threatens the survival of the native species *Scirpus mariqueter*.

Numerous studies, over the last several years, have addressed the issue why *S.alterniflora* has such strong survival competition with *S. mariqueter*. *S. alterniflora* has different ecophysiological properties, such as growth rate, duration of growth over seasons, stem density within canopy, and LAI, from native

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species in Jiuduansha Islands [7]. Also the allelopathic effect of *S. alterniflora* has also been well documented. However, for studying the role of allelopathic compounds in the rhizosphere in the context of invasive plants, activated carbon (AC) has been a widely used approach [8,9]. Activated carbon has an affinity for large organic compounds [10,11]. This factor has led to the use of AC by several studies to remove or immobilize allelochemicals in the soil and several studies have used AC to quantify allelopathic effects already [12,13,14,15,16,17]. However, in the other hand AC may also cause some nutrient interference during it had been added into soil [18]. In this study, we mainly used the media of AC to test the allelopathy of soil invaded by *S. alterniflora*, and then identified the compounds that AC absorbed.

Materials and methods

Materials. The seeds and the soil were both obtained from Jiuduansha Islands. The seeds were collected from monoculture *S. mariqueter* community (N 30°10′12″, E 121°57′40″) while the surface soil (0~20cm below surface) were collected from monoculture *S. mariqueter* community (N 30°10′12″, E 121°57′40″) and monoculture *S. alterniflora* community (N 31°12′35.1″, E 121°58′19.3″). They were labeled as monoculture soil and invaded soil, respectively. All soil samples were allowed to air-dry at room temperature at the laboratory. And then the dry soil was mixed with grit (Φ0.65mm~0.85mm), and this two soil were treated respectively with or without activated carbon. All seeds were imbibed in 0.4% NaCl solution and kept in refrigerator at 4^{o}C to break dormancy until use [19].

*Seed germination bioassay***.** The seeds were sowed in Petri dishes containing different treated soil respectively. So four treatments were conducted: (I) seed + monoculture soil; (II) seed + monoculture soil + AC; (III) seed + invaded soil; (IV) seed + invaded soil + AC. For each sample, 20 seeds were sowed and 10 mL sterile water was added, and each sample was prepared in three replicates. The experiment was conducted in an artificial climate chamber. Germination was determined by counting the number of seeds germinated every 12 hours until there was no new seed germinated for five consecutive days [19].

*Seedling growth bioassay***.** For each treatment, 20 germinated seeds were placed in a Petri dish and added with different treated soil. Each sample was prepared in three replicates and incubated. The incubator light intensity was set to simulate daylight, and the temperature was controlled at 28ºC during the day and 20ºC during the night, and the humidity was maintained at 80%. After 7 days, root length was measured by EPSON Perfection 3200 scanner and analyzed by Regent WinRHIZO (the system of root analysis from Canada).

Extraction of allelochemicals remained in soil. The soil of *S. alterniflora* was soaked in distilled water with AC to oscillate for 48 hours and then AC was removed out. The compounds absorbed by AC was eluted by methanol and then the methanol was evaporated out using a rotary evaporator (Shensheng R-205, Shanghai, China) at 40℃. Samples of extracts was methyl esterification by a solution (methanol: boron trifluoride diethyl etherate=3:1(volume)).

GC-MS. The GC-MS analyses were carried out by using an Agilent 6890N GC system coupled to an Agilent 5975 mass selective detector (MSD) (Agilent, USA). Helium of high purity was used as the carrier gas at a flow-rate of 1 mL·min-1, and the injection volume was 1μL in splitless mode. The GC column used was DB-5MS (30 m×0.25 mm, 0.25 μm film thickness, J & W Scientific, USA). The column temperature was initially held at 50 °C increased to 150 °C at a rate of 5 °C·min^{-1} and further increased to 250 °C at a rate of 15 °C ·min^{-1}, and finally reached 300 °C at a rate of 25 °C ·min^{-1}.

Statistical analyses were performed using SPSS 16.0 statistical software program. All data were evaluated by one-way analysis of variance (ANOVA). From the germination counts the following germination parameters were determined.

(1) Ultimate Germination (UG): The maximum number of seeds that germinated during the experiment [20].

Results

Effects on Ultimate Germination of S. mariqueter. Table 1 showed different treatments significantly affected UGs of *S. mariqueter*. A significant difference in UG among the different treatments was found at confidence level of 95%. The seeds placed in the soil of *S. mariqueter* community had the highest UG (95.00±5.00) %, suggesting that *S. mariqueter* seeds could germinate and grow normally in the original living environment. The lowest UG appeared when the seeds were planted in the soil of *S. alterniflora* community which was (21.67±7.63) % only.

When AC was added in the soil of *S. mariqueter* community, no significant change of UGs was found, however, when AC was added in the soil of *S. alterniflora*, the germination was promoted obviously. The UG of seed in *S. alterniflora* soil with AC was 3.77 times higher than that of seed in *S. alterniflora* soil without AC.

Effects on seedling growth of S. mariqueter. Table 1 showed the effect of different treatment on the seedling growth. The root length was measured in this experiment. Compared the same soil with or without AC, the root length of treatment with AC was distinct higher that of treatment without AC. The root length of seeds sowed in the soil of *S. mariqueter* with AC was (69.48±9.84) cm, while the root length of the same treatment without AC was (19.16±4.00) cm only.

Table 1 Effects of different treatments on *S. mariqueter* germination parameters and seedling growth

Treatments	Ultimate germination[%]	Total root length[cm]
I	95.00±5.00 [a]	19.16±4.00 [b]
II	93.33±2.89 [a]	69.48±9.84 [a]
V	21.67±7.63 [b]	34.60±4.14 [b]
VI	81.67±1.04 [a]	55.83±17.22 [a]

GC-MS. Hexadecanoic acid, octadecanoic acid, dibutyl phthalate, (adipic acid, isohexyl methyl ester) and (adipic acid, di (oct-4-yl ester)) were isolated and identified in soil of *S. alterniflora* by analyzing Fig.1. Among all allelochemicals, some of which were a kind of long-chain fatty acids, and the others were esters. The chemical structures and retention data of the compounds identified were given in Table 2.

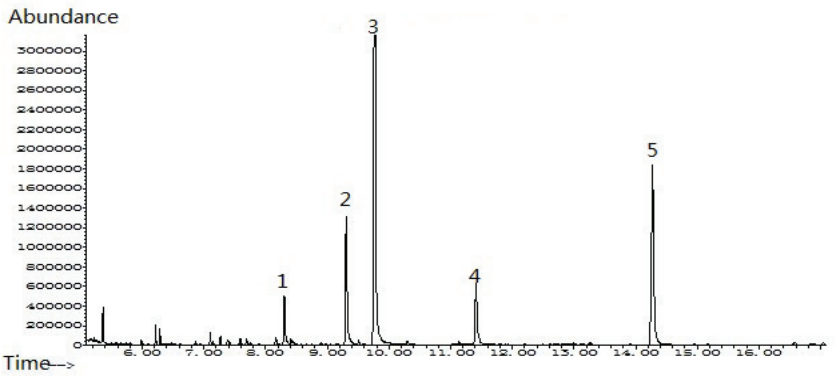

Fig.1 Gas chromatograms of the hydrophobic extracts of *S.alterniflora*

Table 2 the main components in soil through GS-MS

No.	Name	Retention time [min]
1	Adipic acid, isohexyl methyl ester	8.307
2	Hexadecanoic acid, methyl ester	9.309
3	Dibutyl phthalate	9.771
4	Octadecanoic acid, methyl ester	11.415
5	Adipic acid, di (oct-4-yl ester)	14.273

Discussion

Seed germination and seedling stages are considered the most vulnerable periods of the plant life cycle. Poor germination and weak seedlings often lead to limited individual development and species prosperity. It is highly important in plant biology and agriculture practice to study the propagation and adaptability of seeds and seedlings [21].

Our results showed: when the seeds collected from *S. mariqueter* community and their seedlings were planted in the soil from the same community, the seed germination and seedling growth were better than those of other treatments. This indicated that the native species had a high capacity of reproduction and growth in its native environment. This was consistent with the research of Callaway *et al*. [22]. When AC was added in the soil of *S. mariqueter*, the seed germination had basically not affected, however, the seedling growth was promoted distinctly. In recent literatures, it has been suggested that the addition of AC can cause increases in plant growth not because of reduced bioavailability of toxic constituents, but because of additional available nitrogen provided by the carbon itself [18]. And on the other hand, the addition of AC might also change the microorganism in soil which promoted the growth of *S. mariqueter* seedlings. All above indicated the soil of *S. mariqueter* contained no allelochemicals.

In the treatment of seed + invaded soil, the seed germination was seriously inhibited compared to the treatment of seed + monoculture soil, the UG sharply decreased more than 77%, suggesting that the invaded soil inhibited the germination of *S. mariqueter* seeds. But when AC was added in the invaded soil, the UG sharply increased to (81.67±1.04) %. These data confirmed that the soil invaded by *S. alterniflora* may contain its root released compounds, which would inhibit effectively to *S. mariqueter*. Also these compounds might removed by AC so as to reduce the allelopathic effect on seed germination further promote the seedling growth by providing additional available nitrogen [18] or changing the microorganism in soil. According to Weißhuhn's [23], plant available phosphorus increased with AC and this may have caused the better growth of plants.

The allelochemicals sometimes serve as defence chemical weapons to help alien species successfully invade to new areas, and many invasion species release allelochemicals [24].Studies on phytotoxic extracts of Cynodon dactylon showed that the weed extracts contained several phenolic compounds such as ferulic, vanillic, p-hydroxybenzoic and p-coumaric acids [25]. As allelochemicals, long fatty acid and esters were identified in many species, such as in wheat [26], in *Echinochloa crusgalli* [27] and in *Cucumis sativus* L. [28]. In our result, hexadecanoic acid, octadecanoic acid, dibutyl phthalate, (adipic acid, isohexyl methyl ester) and (adipic acid, di (oct-4-yl ester)) were isolated and identified in soil of *S. alterniflora*, most of which have been considered as allelochemicals, and their bioassay might be tested in the further.

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