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Table: 1, Figs : 4

Variations in allelopathic potential among plant species in coastal plant community

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ABSTRACT

We determined the variations in allelopathic potential of plant species in 4-pioneer plant communities (Community *Imperata cylindrica*, Community *Phragmites australis*, Community *Phacelurus latifolius* and Community *Solidago canadensis*) in coastal area in Cixi City, China. The allelopathic potential of species was also compared among functional groups and linked with species dominance. The results showed that main variation in allelopathic potential existed among the species and functional groups. The strength of allelopathic inhibition differed in selected functional groups: annuals/biennials > perennial species, non-clonal species > clonal species and Poaceae < other families. In contrast, the ranking pattern of species mean importance values in different functional groups was reversed. When all species from the communities are pooled together, allelopathic potential was negatively related to the importance value. In conclusion, although the substantial variations in allelopathic potential of plant species exists, but the species dominance in this community was achieved through functional traits viz., clonality and perenniality instead of allelopathy. A trade-off likely exists between traits that promote the dominance and the traits that promote allelopathy.

Key words: Allelopathy, allelopathic potential, functional group, importance value, plant community, plant invasion, strength of inhibition

INTRODUCTION

Many studies have addressed the significance of allelopathy in ecological questions. Among these questions, how populations are organized into higher units or “communities” is major issue in ecology (13). The ecologists have recognized the importance of chemical interactions (Allelopathy) between the plant species (3) and it was hypothesized that adaptation of plant species to the chemistry of other species was crucial to this organization (18). However, due to the complexity of allelopathy, there are no convincing conclusions of its ecological significance in modification of plant community structure. Few studies have explored the variations in allelopathic potential in the plant community (2,14,16,17). These studies compared the allelopathic potential of species among functional groups with different origins (invasive or non-invasive), life forms (from herbs to trees) and families and also related allelopathic potential of species to other

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important functional and ecological traits (Plant height, species cover in field) to determine the role of allelopathy in plant community development. However, allelopathy is such a complex process that its role in the plant community still remains unclear and must be further elucidated (26). This study aimed to provide the basic information on variations in allelopathic potential among the plant species and functional groups (Invasive species vs. non-invasive species, clonal species vs. non-clonal species, etc.) in a coastal pioneer plant community. This information is important to further explore the significance of allelopathy to regulate the dynamics of plant community. We also related the allelopathic potential in plant species to dominance in the field, to provide the additional evidence of relationship between allelopathy and plant community development.

METHODS AND MATERIALS

Study site

The studied area (center: 121°09’58” E, 30°19’29” N, Mean height above sea level: 1 m) was originally part of tidal plains and was separated from the Hangzhou Bay in 1997, when a dike was built. Therefore, the direct influence of tidal seawater was eliminated. Thereafter, the land was used as agricultural fields until 2009, when a national wetland park was set up. In the national park, the area has been strictly protected from human disturbance and the vegetation began as secondary succession. The soil water content and salt content gradually decreased after disconnection with tidal plain. Presently the soil is coastal saline and silty clay in texture. The mean soil water content is 14.9-21.5% and salt content is 0.1-0.5%. The soil pH is above 8, and the average available soil N, P and K contents are 47.3, 5.7 and 92.1 mg/kg, respectively. The area lies in the subtropical monsoon zone, hence, the climate is mild, warm and humid. The duration of summer (June to September) and winter (December to March) is longer than spring (April to May) and autumn (October to November). The annual mean air temperature is 16.0^{o}C and is lowest (3.8 ^{o}C) in January, Mean rainfall: 1344.7 mm, with peaks in June and September and annual average sunlight duration: 2038.4 h and the frost-free period: 244 days.

Plant community and species dominance

In the abandoned agricultural fields we found 4-pioneer communities with different dominant species (Table 1): (i) *Imperata cylindrical* (*Poaceae*) Community, (ii) *Phragmites australis* (*Poaceae*) Community, (iii) *Phacelurus latifolius* Community and (iv) *Solidago canadensis* (*Asteraceae*) Community. These all communities have developed in the coastal salt marshes dominated by *Scirpus mariqueter* (Cyperaceae), *P. australis* (Poaceae) and *Spartina alterniflora* (Poaceae) with *Suaeda glauca* (Chenopodiaceae). The vegetation is strictly protected.

A stratified sampling method was used in the field survey (6), according to the four types of vegetations with the different dominant species listed above (Table 1). Quadrats (1×1 m) determined by square frames were used for vegetation sampling. In each community, the plot location was randomly determined and the number of sampling plots for each community was proportional to its area. A total of 176 quadrats were surveyed,

Table 1. Characteristics (dominant height, coverage, plant density and major accompanying species) of 4-dominant communities

Community type	Dominant species height (m)	Coverage (%)	Plant density (plants/ m^2)	Major accompanying species
I. cylindrical	0.65	85.1	148	*Melilotus officinalis*, *Artemisia lavandulaefolia, Sonchus brachyotus*, *Erigeron annuus, Metaplexis japonica*, *Solidago canadensis*
P. australis	1.83	66.8	37.9	*Carex scabrifolia, Aster subulatus, Polypogon fugax*, *Sonchus brachyotus, Solidago canadensis*
P. latifolius	1.92	81.9	51.4	*Artemisia lavandulaefolia*, *Glycine soja, Erigeron philadelphicus*, *Sonchus brachyotus*, *Imperata cylindrica*
S. canadensis	1.43	85.1	62.8	*Artemisia lavandulaefolia*, *Imperata cylindrica*

and in each plot were recorded the coverage, shoot height and species abundance. As many of these species are clonal plant species, the ramet (the physiologically distinct plant that is part of a group of genetically identical plants derived from one progenitor) was considered as the basic unit to record the species abundance. All ramets from a single plot were harvested and counted. The dominance of each species was indexed by the importance value, calculated as per Curtis and McIntosh (1),

$$\text{Importance value (IV)} = D_r + C_r + F_r$$

Where D_r : Relative density, C_r : Relative coverage and F_r : Relative frequency. These values are calculated as under:

$$D_r = \frac{D_i}{\sum D_i} \times 100 \text{ , } C_r = \frac{C_i}{\sum C_i} \times 100 \text{ , } F_r = \frac{F_i}{\sum F_i} \times 100$$

Where, D_i , C_i and F_i are density, coverage and frequency, respectively, of the ith species in a community.

Allelopathic potential

In this study, we used the bioassay approach was used instead of detailed and chemically-focussed autecological approach. Compared with the latter approach, the bioassay approach provides several advantages: (i). It reflects all chemicals rather than particular substances produced by a plant species; (ii). It is effect-based under controlled conditions, hence, diverse species can be compared, despite their different chemical properties, life forms and habitats and (iii). It is less laborious, if large numbers of species are to be compared (14). Due to these advantages, this approach is very suitable for allelopathy assessment at the community level.

Plant material collection: Leaf material of 35 species was collected in August 2014, during the grand growth phase of most species. These species represent wide range of species origins (native and non-native), life forms (annual/biennial and perennial), clonality (clonal and non-clonal) and different taxa and the species currently abundant to collect their sufficient leaf material. Leaf tissues were collected from several healthy and vigorous individuals of each species. In most cases, leaf tissues were collected from 10 or more individuals to capture any variation among individuals in allelopathic activity. Because of their small sizes, the number of individuals collected from many of the herbaceous species was much larger to provide sufficient leaf material. Collected leaf material was placed in paper bags and immediately dried at 60℃ within 24 h to prevent microbial breakdown of tissues. A single target species and leaf tissue were used for logistical reasons to allow for the comparison of a large number of plant species.

Leaf extract bioassay: The allelopathic potential of each plant species was assessed using the modified bioassay method by Meiners (14). Chinese cabbage (*Brassica chinensis*) was used as the target species in all trials because it germinates quickly, is sensitive to allelopathic inhibition and can differentiate among the species. For each specie, the leaf blades were cut into 3 cm long pieces and the extracts were prepared from 6.25 g dried leaf tissue in 250 ml of deionized water. The mixture was placed on magnetic stirrer for 24 h at room temperature and strained through cheesecloth to remove particulate plant material. The extracts were diluted with deionized water to concentrations of : 0, 10, 20,30,40,50, 60, 70, 80, 90 and 100% of the original concentration. Two filter papers were placed in 90-mm petri dishes, then 30 seeds of target species were kept on top of filter paper. The treatments were replicated thrice in Randomized Blocks Design. Five mL extract was added to each plate only once and incubated at 25 ℃ under a 12/12 h light/dark cycle Petri dishes were placed in sealed plastic bags to retain moisture. The *B. chinensis* seeds germinated 4 days after incubation. The germinated seeds were counted and the radical length of each seedling was measured on the fourth day after incubation.

Statistical analysis

The radical length was analyzed via linear regression using the averaged data from each dish individually as a function of extract concentration (see the example of Fig. 1). The coefficient from this analysis was used as the metric for allelopathic potential and is defined as strength of inhibition. Afterwards, the mean value, standard deviation and frequency distribution of species strength of inhibition were analyzed. The importance value and strength of inhibition of the species from different functional groups: families life forms (perennial vs. annual/biennial species), clonality (non-clonal vs. clonal species) and originality (non-native vs. native species) were averaged, and the means difference between different groups were tested. Correlation between the species strength of inhibition and the importance value of the corresponding species was measured with Pearson’s correlation. Those species that occurred only once in all surveyed quadrats were neglected in the analysis. All the analysis was conducted in SPSS 16.0.

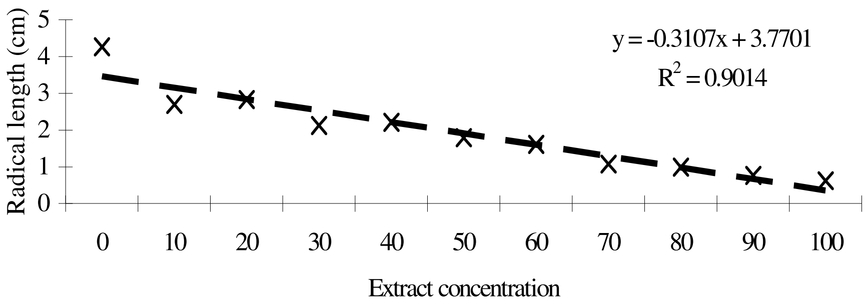

Figure 1. An example of linear regression of the relative leaf extract concentration (% of the original concentration) against the radical length of germinated *Brassica chinensis* seeds. Strength of inhibition was measured as the absolute value of the regression coefficient from this regression for germination in each species. The crosses represent radical length data measured at the corresponding relative extract concentrations and the broken line represents the calculated value from the linear regression model.

RESULTS AND DISCUSSION

Allelopathic potential among species and functional groups

In the selected 41 species, leaf extracts did not influence the seed germination of *B. chinensis* (data not shown), but the radical length of seedlings was influenced. Except *I. cylindrical*, the radical length of germinated *B. chinensis* seeds exposed to leaf extract from other species was negatively correlated with leaf extract concentration. The coefficient of this correlation, referred to as the strength of inhibition, varied remarkably with the donor species. Therefore, this value can be used as a representative of allelopathic potential of leaf extracts. Substantial interspecific variation (n = 41, standard deviation = 0.1184) of allelopathic potential occurred. The inhibition strength ranged between 0.0075-0.4911 with mean value of 0.2554. The distribution curves of inhibition strength of all tested species did not follow a normal distribution but rather moved rightward (skewness = 0.057, Fig. 2). A question is what pattern of variation of species allelopathic potential was in the plant communities? Meiners (14) demonstrated that allelopathic potential is linked with other species functional traits, such as life span, plant height and leaf mass, etc. In accordance with these findings, we also observed that the species mean alleloapthic potential differed significantly among the functional groups. The species allelopathic potential from *Poaceae* were significantly lower than *Asteraceae*, *Leguminosae* and other families as a whole ($P < 0.05$), whereas, the later three groups were not significantly different from each other ($P > 0.05$, Fig. 3A). In contrast, species from *Poaceae* displayed the highest importance value, which was significantly higher than other families ($P < 0.05$), except for *Asteraceae* and *Leguminosae* (Fig. 3A). For different life forms, the perennials had significantly lower allelopathic potential than annuals and biennials ($P < 0.05$), but the perennials were more dominant than the annuals and biennials, although the differences were non-significant ($P = 0.086$) (Fig. 3B). When the species were grouped into clonal species and non-clonal species, the allelopathic potential was significantly lower ($P < 0.05$) and the importance value was significantly higher in clonal species ($P < 0.05$) than in the non-clonal species (Fig. 3C).

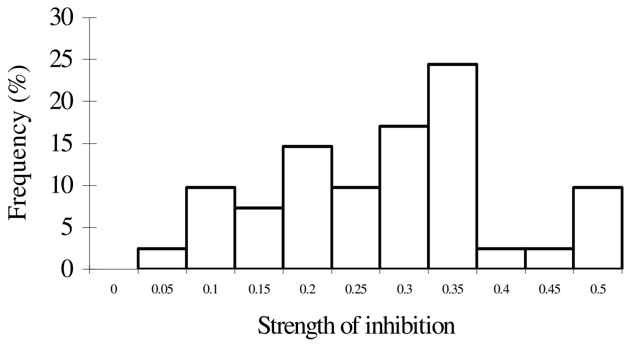

Figure 2. Frequency distribution of strength of inhibition of the *Brassica chinensis* seed radical length by the leaf extracts of the 41 donor plant species.

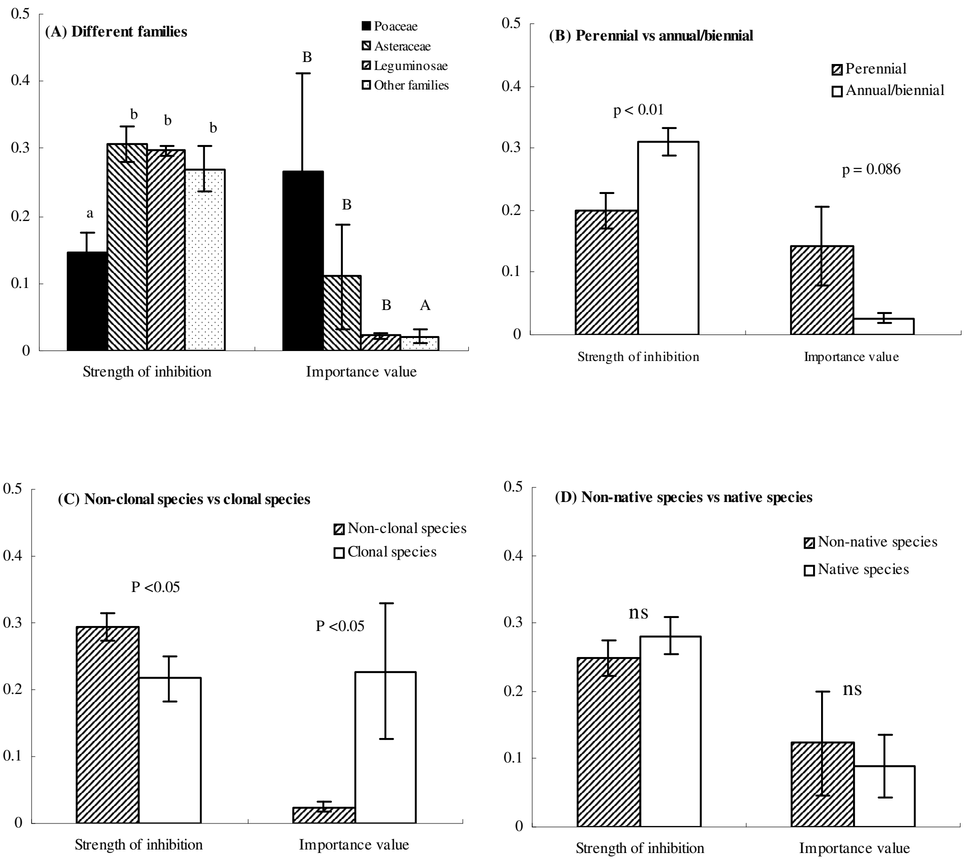

Figure 3. Mean allelopathic potential and importance value of different functional groups. If P < 0.05, the difference between various functional groups was considered significant; different letters indicate significant difference, and ns indicates non-significant.

In our study, the allelopathic potential was marginally lower (Fig. 3D, P > 0.05) and the importance value was marginally higher (Fig. 3D, P > 0.05) for the non-native species than native species. Correspondingly, the inhibition strength of *S. canadensis*, a dominant and highly invasive species, was only 0.2605, which was much lower than certain other native species (e.g., 0.456 for *Trifolium vulgare* and 0.415 for *Eclipta prostrata*, both from *Asteraceae*). This result did not support the hypothesis that exotic species exert their dominance through high allelopathic potential. Similar results were also reported by Pisula and Meiners (17) and Meiner (14). Wardle *et al* (23) suggested that allelochemical production and activity in invasive species might decline over time after their introduction into new regions because resident species and invasive species might co-adapt to each other in an evolutionary manner.

Correlation between plant species dominance and allelopathic potential

The direction of the variation of allelopathic potential and importance value between different functional groups was reversed (Fig. 3). Furthermore, if all species within each community are pooled together, the species allelopathic potential and importance value were negatively correlated in all four communities (Fig. 4), althogh the correlation was significant only in *I. cylindrical* community (Fig. 4D, P < 0.01). Moreover, when all species from the four communities were pooled together, the correlation between allelopathic potential and importance value was significant (Fig. 4E, P < 0.05). All these results suggested that a trade-off probably exists between the traits that promote dominance and allelopathy.

What are the possible explanations for the negative correlation between the species allelopathic potential and dominance? Because most of the allelopathic substances are synthesized in secondary metabolism and are costly to produce, store and transport, resources must be carefully allocated to get profits from these costs. It is well accepted that the ability to compete for resources is most important trait that determines the species distribution and abundance (4,18). Therefore, the traits that promote competition ability might compete for limited resources with traits associated with allelopathy (25). In our case, these traits probably include perenniality and clonality (Fig. 3). Because perennials are already settled in specific habitats, it is probably more effective in species interaction for the perennials to promote vegetative growth than to chemically suppress their neighbors, whereas annuals must be very allelopathic to acquire the space and resources at the start of growth season. Similarly, clonality promotes integration and labor division between ramets, and therefore, clonal plants are more effective in utilizing limited resources and benefit from higher vegetative growth rates (10,21). The faster growth rate of clonal plants demands that additional resources are used for vegetative growth and consequently, fewer resources are allotted to secondary metabolism activities, which results in fewer allelopathic substances produced and released into environments. This observation is in accordance with the finding that clonal species have lower allelopathic potential than non-clonal species (Fig. 3C). The trade-off between plant growth and secondary metabolism has been verified in other research work (7,20).

The absence of a positive correlation between allelopathic potential and species dominance suggests that allelopathy might be not as important as was assumed (2,15). Reigosa *et al* (19) noted that the influence of allelopathy cannot be significant as a

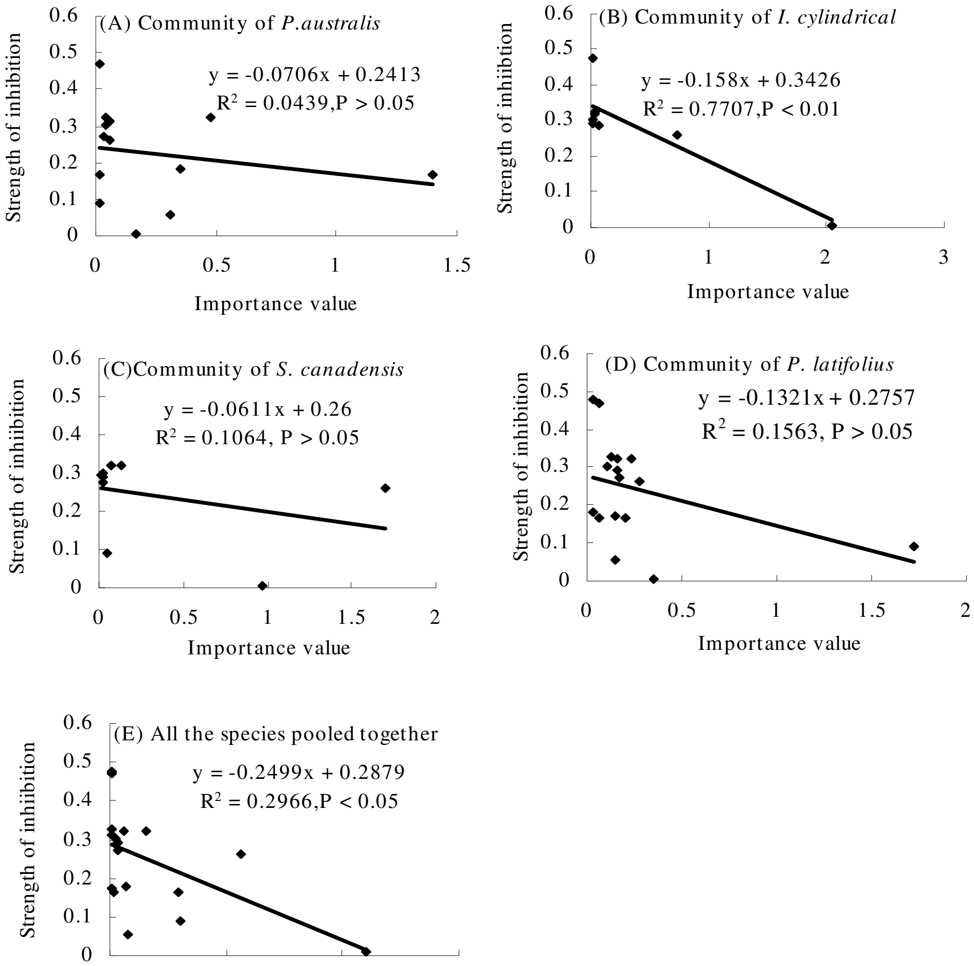

Figure 4. Regression of importance value against species allelopathic potential (indexed by strength of inhibition) in four plant communities: Community *I. cylindrical*, Community *P. australis*, Community *P. latifolius* and Community *S. canadensis*.

universal process due to the co-evolution of the residents within a community. The conditionality of allelopathy in the field has been reported (11,19). However, plants with allelopathic potential are more likely to have wide-ranging influences at the ecosystem level rather than at an individual level through production of phytotoxins (24). In addition to its role in direct interactions between species, allelopathy might affect the plant communities via much more complicated ecosystem processes, i.e., plants inhibit or facilitate neighbours by releasing allelochemicals, and they can also indirectly influence the neighbor species by modifying biotic and abiotic interactions, such as nutrients uptake and bioavailability (24,26). Furthermore secondary metabolites, which can be allelopathic only when they are released into environments, are more often deployed as weapons against the diseases and herbivores (4) and thus, are significant in plant--herbivore/pathogen interactions. In short, the relationship between the allelopathy and dominance cannot be simply explained by an effect-consequence pattern.

Several limitations of the protocols applied in this study must be noted. Because of the type of allelochemicals produced, the amounts of these chemicals and the recipient species could all significantly affect the allelopathic interactions among the plant species, and potential bias could occur in quantifying the allelopathic potential of selected donor species. The recipient specie (*B. chinensis*) used in this study is not a wild species that grows naturally in the studied plant community. This crop species might respond differently to the tested plant species extracts from those species in the plant community. Secondly, laboratory bioassays only provide preliminary information on species allelopathic potential (8,12) because it does not consider the interaction effects of various complex soil components and soil processes with allelochemicals (9). However, although serious conclusions cannot be drawn without further careful examination of the role of allelopathy in this plant community construction, this study at least provides the preliminary evidence and groundwork for further studies.

Our results reinforces the idea that allelopathy is a complex process. Due to the limitation of the protocols applied in this study, detailed studies that are conducted under the more natural conditions and that investigated the mechanisms of allelopathy are strongly needed in the future.

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