Influence of invasive plant Coreopsis grandiflora on functional diversity of soil microbial communities

Abstract

Biological invasions are increasingly attracting the ecologists’ attention. Invasive plants threaten the natural ecosystems not only by competing with the native plants, but also by altering the structure and function of soil microbial communities belowground. In this study, we studied the effects of the invasive plant Coreopsis grandiflora on the functional diversity of soil microbial communities in Laoshan mountain in the province of Shandong, North of China. We sampled soil from plots that were invaded or not invaded by C. grandiflora. The functional diversity of microbial communities in the sampled soils was assessed by the Biolog procedure test. By the ANOVA analysis of average well color development (AWCD), Shannon index ($H'$), Shannon evenness ($E$), principle components analysis of the level physiological profiles (CLPP) and correlation analysis between the studied parameters, we found that the invasive species C. grandiflora enhanced the functional diversity of soil microbial communities where the habitat was invaded by the C. grandiflora. The study indicated that the successful invasive plants have profound effects on the function of soil microbial communities.

Key words
Biolog, Biological invasions, Carbon substrate consumption, Coreopsis grandiflora, Environmental tolerance, Soil microbial community

Introduction

Biological invasions have threatened natural ecosystems throughout the world (Callaway et al., 2004; Liu et al., 2005; Wang et al., 2008; Straube et al., 2009) and have been recognized as a global environmental problem (Singh and Mishra, 2001; Ou et al., 2008). A major focus of studies on plant invasions is describing the effects of invasive species on native communities as well as the attributes of native communities that make them susceptible to invasion (Wolfe and Klironomos, 2005). Invasive plants alter the original biological characteristics of the communities and ecosystems, such as species composition in the community, and plant productivity. These effects often lead to the decrease of the biological diversity on the local and regional ecosystems, and threaten the structure and function on the natural ecosystems (Bertness, 1984; Vitousek, 1990; Xu et al., 2007; Goldstein et al., 2009; Wan et al., 2009).

Most researches on the effects of the invasive plants have focused on aboveground flora and fauna (Dutta and Agrawal, 2005; Wolfe and Klironomos, 2005; Chen et al., 2009; Wang et al., 2009; Xu et al., 2010). Effects on belowground flora and fauna have been
rarely studied. Rhizosphere, the soil surrounding plant roots, possess a unique physical, biochemical, and ecological conditions and is to a great extent controlled by the root system itself through chemicals exudation and secretion (Bais et al., 2002, 2003). Invasive plants can change the rhizosphere environment by secondary compounds from root and the quantity, quality, and timing of litter inputs. Although microbial communities may be altered as the result of the plant invasion, the effects of invasive plants on the function of soil microbial communities appear highly variable, partially because the effects were only documented by a handful of studies (Wolfe and Klironomos, 2005). The observed effects on the function of soil microbial communities varies depending on the invasive plants studied, the community or ecosystem invaded, the methods used to assess changes and the temporal and spatial scales considered in the study (Roberts and Anderson, 2001; Kuske et al., 2002; Duda et al., 2003; Wolfe and Klironomos, 2005). Thus, more studies are needed to understand the effects of the invasive plants on the function of soil microbial communities.

The function of soil microbial communities is closely linked with composition and function of aboveground vegetation (Wardle et al., 2004), so the invasive plants can directly and/or indirectly disrupt the established links between native plants and soil microbial communities (Wolfe and Klironomos, 2005). Invasive plants may change ecosystem function via altering soil microbial communities (Marchante et al., 2008, 2009). Soil microbial communities play important roles in regulating ecosystem-level process, including nutrient cycling, decomposition of organic matter (Zeng et al., 2005). Soil microbial communities changed by the invasive plants will cause the unexpected results in the natural ecosystems. Soil microbial communities also have strong effects on exotic invasive plants. Plant-soil feedbacks may differ between some exotic and native species (Callaway et al., 2004). Therefore, the relationships between soil microbial communities and invasive plants are hotspots for exotic species (Callaway et al., 2004; Reinhart and Callaway, 2006; Mangla et al., 2008; Cui and He, 2009).

Coreopsis grandiflora Hogg. (C. grandiflora) was introduced to China from the North America as an ornamental flower, which was cultivated in the botanical gardens all over the nation. No literature recorded the exact time when C. grandiflora was introduced and now it has become one of the main invasive plants in China (Ministry of Environmental Protection of the People’s Republic of China, 2002; Liang et al., 2008). C. grandiflora has a broad geographic distribution in North America and the East Asia. In Shandong Province, China, it diffusively distributes in the field, in particular, in some coastal cities such as Qingdao, Weihai (Tian, 2004), and display high genetic diversity within populations in Laoshan Mountain (Liang et al., 2008). The species has strong reproduction ability by seeds and rhizome, and mostly distributes in the edge of the forest with high irradiance. The pilot study found that the invasion of C. grandiflora had significant influence on the diversity of plant communities and the mechanism is not clear, the invasion of C. grandiflora may have profound effect on the soil biota.

We studied the effects of the invasive plant C. grandiflora on the functional diversity of soil microbial communities in Laoshan mountain (Shandong Province, North of China).

**Materials and Methods**

**Field sampling:** The field sampling was conducted on Laoshan mountain in the April, 2007. Laoshan mountain, a famous beauty spot, is the highest mountain along the coast of China. Four sites were selected for the sampling. The four sites were located at the different altitudes in Laoshan mountain (site A: 262m, 36°22’N, 120°59’E; site B: 271m, 36°22’N, 120°60’E; site C: 360m, 36°21’N, 120°60’E; site D: 320m, 36°21’N, 120°60’E). Site A was flat and located near a river, while site B, C and D were steep and near pinewoods in the hills. In each site, we selected five 1 x 1m sampling plots where grow C. grandiflora. In addition we selected three 1 x 1m sampling plots where C. grandiflora not invaded as control. The control plots are adjacent to the plots where grow C. grandiflora to ensure the similar environmental conditions. There is a 5m interval between the sampling plots. We collected 3 repeated soil samples (about 500-800 g) from the top 10 cm of the soil profile in each sampling plot and got 96 soil samples in all. The soil samples were immediately placed in sealed plastic bags and transported to the lab in an icebox for further analysis. The soil samples from site B, C, D contained more sand than site A. After being sieved through 2 mm mesh, all soil samples were treated for following analysis within one week after field collection.

In each sampling plot, we recorded the coverage of C. grandiflora, the species richness, the coverage of the aboveground native species. The coverage of native plants in the most control plots was less than 10% because of the growth of native plants is later than C. grandiflora.

**Measurement of functional diversity of microbial community:** Functional diversity of soil microbial communities was assessed by the Biolog procedure. Biolog analysis was used to assess the function of soil microbial communities. The Biolog analysis is predicated on the idea that different species have different capacities to metabolize a range of simple substrates (sugars, amino acids, fats etc.) and so the response of whole soil communities to such a range of substrates is an indicator of the diversity of soil microbial community present and their potential functional capabilities (Kourtev et al., 2002). A subsample (10 g) from each soil sample was dissolved in 100 ml sterile saline (0.85 mol l⁻¹ NaCl). After 10 min of suspending, the supernatant was recovered. Soil solution was obtained by diluting supernatant with 100 times finally. Then, 150 μl aliquots of soil dilution were inoculated to ECO-Biolog plates. The plates were incubated at 28°C darkness for 144 h. The absorbance values of ECO-Biolog wells were read at 590 nm every 12 hr during the culture using Microlog™ Release 4.20 software (ML3402, Microlog, USA) (Han et al., 2007a,b; Zhang et al., 2008).

**Data analysis:** For community level physiological profiles (CLPP)
Influence of invasive plant on functional diversity of soil microbial

Table - 1: The coverage of C. grandiflora and native species of the sampling plots.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Plots</th>
<th>The coverage of C. grandiflora %</th>
<th>The species richness</th>
<th>The coverage of native species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site A</td>
<td>Plot 1</td>
<td>0.309±0.137</td>
<td>4.60±0.548</td>
<td>0.038±0.015</td>
</tr>
<tr>
<td></td>
<td>Plot 2(control)</td>
<td>-</td>
<td>3.33±0.577</td>
<td>0.077±0.015</td>
</tr>
<tr>
<td>Site B</td>
<td>Plot 3</td>
<td>0.352±0.252</td>
<td>4.20±2.280</td>
<td>0.026±0.018</td>
</tr>
<tr>
<td></td>
<td>Plot 4(control)</td>
<td>-</td>
<td>7.00±1.000</td>
<td>0.087±0.03</td>
</tr>
<tr>
<td>Site C</td>
<td>Plot 5</td>
<td>0.214±0.161</td>
<td>5.60±2.302</td>
<td>0.06±0.019</td>
</tr>
<tr>
<td></td>
<td>Plot 6(control)</td>
<td>-</td>
<td>5.66±1.528</td>
<td>0.083±0.025</td>
</tr>
<tr>
<td>Site D</td>
<td>Plot 7</td>
<td>0.224±0.0804</td>
<td>3.00±1.000</td>
<td>0.042±0.029</td>
</tr>
<tr>
<td></td>
<td>Plot 8(control)</td>
<td>-</td>
<td>3.66±1.528</td>
<td>0.073±0.015</td>
</tr>
</tbody>
</table>

Plot 1, 3, 5, 7 were invaded by the invasive plant C. grandiflora, and plot 2, 4, 6, 8 were not invaded by C. grandiflora. Each value represents the mean ± SD.

Table - 2: Two-way ANOVA with repeated measurements for the effects of different sites and C. grandiflora on the AWCD, Shannon index and evenness. Site and invasion status were used as two main factors, and time was used as the repeat term.

<table>
<thead>
<tr>
<th>Effect</th>
<th>AWCD</th>
<th>H'</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>1.943***</td>
<td>7.744***</td>
<td>3.273*</td>
</tr>
<tr>
<td>C. grandiflora</td>
<td>18.081***</td>
<td>36.238***</td>
<td>21.202***</td>
</tr>
<tr>
<td>The interaction effect</td>
<td>9.024***</td>
<td>4.145**</td>
<td>5.568**</td>
</tr>
<tr>
<td>Site and C. grandiflora</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Each value represents F significance level. Significance level: ***: p < 0.001; **: p < 0.01; *: p < 0.05.

Substrate utilization and functional diversity of soil communities: AWCD, H' and E had significant differences between the different incubation time. Sites had no effects on AWCD, while sites had significant effects on H' and E. The colonization of C. grandiflora had significant effects on AWCD, H' and E. The interaction effect of site, invasion showed significant effects on AWCD, H', E (Table 2). The response of functional diversity of soil communities to C. grandiflora at different sampling sites was different, so the effect of C. grandiflora on soil microbial functional diversity is site specific.

The study found significant effects of the plant invasion on the functional diversity of soil samples. The significant differences of AWCD, H', E were founded by the one-way ANOVA (p<0.01). The Duncan test was used to compare AWCD, H', E of 72 and 144 hr among the different plots (Fig. 1). AWCD of 72 and 144 hr at plot 1, 2, 3, 4, 5 were significantly higher than plot 4, 6, 8. The higher values of AWCD mean the soil communities have the higher capacities to metabolize a range of simple substrates and reflect the higher diversity of soil microbial community. H' of 72 and 144 hr at plot 1, 2, 3, 4, 5 were significantly higher than plot 4, 6, 8. AWCD and H' with C. grandiflora were significantly higher than the control plots except for the plot 2 E' of 72 hr at plot 1, 2, 3, 4, 5, 7 were significantly lower than plot 6, 8 E' of 144 hr at plot 1, 2, 3, 5, 7 were significantly lower than plot 6, 8.

The first two principal components (PC) of the CLPP explained 65.04 % of total variance with PC1 accounting for 54.8% (Fig. 2a). The first principal component (PC1), which is associated with soil carbon source usage, separated the soil samples into two groups: the plot 1, 2, 3, 5, 7 and the plot 4, 6, 8 (control) (Fig. 2a). Plot 1, 2, 3, 5, 7 tended to have greater utilization of most of the carbon sources, whereas the plot 4, 6, 8 had less use of most of the carbon sources. For PC2, the important carbon sources were C10, C11 and C16 in the negative direction. For PC2, the important carbon sources were C10, C11 and C16 in the negative direction.

Results and Discussion

The field sampling result: The mean coverage of C. grandiflora in plots C. grandiflora invaded was from 0.224 to 0.352, the mean coverage of native plants in plots C. grandiflora not invaded was from 0.073 to 0.087, the species richness and the coverage of the native species in plots C. grandiflora invaded was lower than those in plots C. grandiflora not invaded (Table 1). C. grandiflora which invaded Laoshan Mountain decreased the local native plants diversity.

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utilization of most of the substrates than the control samples except for samples of plot 2.

The study found significant effects of the invasive plant *C. grandiflora* on the functional diversity of soil microbial communities and the effects was site-specific. Our results also showed that the response of soil microbial communities’ functional diversity to the invasion of *C. grandiflora* was obviously different between site A and site B, C, D. Site A was flat and located in the floor of river. Site B, C, D were on steep hills which was the common habitat in the Laoshan Mountain. The soils in site A are more convenient to obtain water than soils in other sites. The soils from site B, C, D contained more sand than site A. Although the low coverage of native plants in all the control samples, the roots in the soils from plot 2 were much more than other control plots. Because of more roots and less sand in the soil, the soils in site A might be higher in the concentration of some essential nutrient elements such as N, P and K. Because the properties of the soil have strong influence on the soil communities (Singh et al., 2009), the effects of invasive plants on the functional diversity of soil microbial communities may vary with local environmental conditions.

**Fig. 1:** AWCD, Shannon index ($H'$) and evenness ($E'$) of soil microbial community between sampling plots. a: 72 hr AWCD; b: 144 hr AWCD; c: 72 hr $H'$; d: 144 hr $H'$; e: 72 hr $E'$; f: 144 hr $E'$. The different lower case letters represent a significant difference among the treatments in each species ($p<0.05$, Duncan’s test). Bars indicate one standard error of the mean (n=15 for the plot invaded by *Coreopsis grandiflora*, n=9 for the plot where *C. grandiflora* not invaded).

**Fig. 2:** Principal components analysis (PCA) of Eco-Biolog plates profiles from microbial communities of soil samples (a). Loadings of Biolog substrates in PCA. C1 to C31 were the 31 kinds of different sole carbon source (b). PC1 (54.80%) separated the soil samples into two groups: the plot 1, 2, 3, 5, 7 and the plot 4, 6, 8 (control). Plot 1, 2, 3, 5, 7 tended to have greater utilization of most of the carbon sources, whereas the plot 4, 6, 8 had less use of most of the carbon sources.

**Relationship between the coverage of *C. grandiflora*, total coverage and AWCD, Shannon index, evenness of soil samples:** The coverage of *C. grandiflora* were positively correlated with AWCD and $H'$ of 72 and 144 hr ($p<0.01$), while the coverage of *C. grandiflora* were negatively correlated with the diversity of the soil microbial communities in terms of the index $E$ of 72 and 144 hr ($p<0.01$). The invasive plant *C. grandiflora* enhanced the capacities of soil microbial communities to metabolize simple substrates, but the capacities to metabolize different substrates were different. The soil communities at the influence of *C. grandiflora* had higher capacities to metabolize *Tween* 40, *Tween* 80, $\beta$-Methyl-D-glucoside, D-Xylose, D-Mannitol, N-Acetyl-D-glucosamine, D-Cellobiose, $\alpha$-D-Lactose, L-Arginine, L-Asparagine than other carbon sources, so the coverage of *C. grandiflora* was negatively.
Influence of invasive plant on functional diversity of soil microbial communities correlated with the diversity of the soil microbial communities in terms of the index E.

The response of soil communities with C. grandiflora was consistent in these 3 sites. The low coverage of native plants and fewer roots in the soils indicated the native plants had difficulties to grow on the steep hills. C. grandiflora has no strict requirement for soil. The tolerance to environmental factors facilitates the exotic plants invasion (Sutherland, 2004; Kuster et al., 2008). C. grandiflora can invade the habitat which was not suitable for the natives on the mountains and encounter less resistance because of low diversity of native plant communities. The higher tolerance to poor soil conditions may be one important trait in the invasion of C. grandiflora. The presence of C. grandiflora promotes the functional diversity of soil microbial communities.

When the field sampling was conducted, the mean coverage of C. grandiflora in plots C. grandiflora invaded was from 0.224 to 0.352, the mean coverage of native plants in plots C. grandiflora not invaded was from 0.073 to 0.087. The earlier phenology of invasive plants in the habitats, will not only change the initial function of soil microbial communities as the results in the study (Ehrenfeld et al., 2001; Li et al., 2006), but also change the structure of soil microbial communities (Kourtev et al., 2002, 2003; Angeloni et al., 2006; Li et al., 2006). The relation between the invasive plants and the soil function is very complex and need further study.

The feedback of changed soil microbial communities may cause the unexpected results and the native soil microbial communities may in turn hasten invasional meltdown by benefiting the exotic plants and suppressing the native plants (VanderPutten and Peters, 1997; Richardson et al., 2000; Hart et al., 2003; Callaway et al., 2004; Grosholz, 2005; Niu et al., 2007; Kulmatiski et al., 2008; Zhang et al., 2009). The growth of C. grandiflora in relatively poor soil conditions may elevate the nutrition by the activities of soil communities and the roots or further reduce the nutrition by the absorption. The invasive plant firetree (Myrica faya) in Hawaii was found to affect nitrogen cycling and plant community composition in these communities (Vitousek and Walker, 1989). Both management of invasive plants and the restoration of native plants must consider the soil environment changes (Kourtev et al., 2003). The influence of C. grandiflora on the functional diversity of soil microbial communities is very important to the invasion process of C. grandiflora.

Our study affirmed that the invasive species C. grandiflora enhanced the functional diversity of soil microbial communities in Laoshan Mountain region. The study indicated that the successful invasive plants have profound effects on the soil microbial communities and need further study.

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