Effects of species richness on cadmium removal efficiencies of algal microcosms

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Summary

1. An important factor limiting the wider application of constructed ecosystems for bioremediation of sites contaminated by toxic chemicals is their relatively low efficiency of contaminant removal. Although there is growing evidence that species-rich ecosystems may out-perform species-poor ecosystems in removing excessive nutrients from water through niche partitioning, it remains unknown whether diverse ecosystems are more efficient in removing toxic chemicals from the environment, and if they are, by what mechanisms diverse ecosystems can lead to enhanced removals.

2. In this study, we exposed aquatic algal microcosm ecosystems of varying species richness to a realistic cadmium (Cd) contamination scenario. We explore the mechanisms of Cd removal by assemblages with differing diversity and consider the potential role of diversity on Cd bioremediation.

3. Our results suggest that Cd removal efficiencies of the algal microcosms increased with species richness. Furthermore, we found that 45% of all polycultures out-performed the monocultures of their most efficient component species in removing Cd from the growth substrates (referred as to ‘over Cd removal’). However, the average Cd removal efficiency of the most diverse (eight-species) polycultures was not higher than that of the most efficient monoculture (i.e. the algal species most tolerant to Cd) in this study. We also showed that the observed over Cd removal could be largely ascribed to the enhanced biomass yields of the polycultures, which were mainly driven by the positive effects of Cd-tolerant species on Cd-sensitive species.

4. Synthesis and applications. This study demonstrates that some algal polycultures containing both Cd-tolerant and Cd-sensitive species may show over Cd removal through facilitation provided by Cd-tolerant species. These polycultures are likely to be assembled and applied to achieve Cd removals higher than those of their most efficient component species in monoculture. Given that species-rich ecosystems tend to be more environmentally stable than ones with fewer species, it would be prudent to employ diverse polycultures rather than risk the loss of individual monocultures.

Key-words: algae, biodiversity, bioremediation, heavy metal, interspecific facilitation, restoration

Introduction

Sites contaminated by heavy metals or other toxic chemicals are common throughout the world today, and the exposure to these pollutants through the food chain may lead to serious ecological problems (Larison et al. 2000). Therefore, there is an urgent need to detoxify or restore these polluted ecosystems. Because of its relatively low capital cost and inherently aesthetic nature, bioremediation, defined as any process that uses living organisms to detoxify or restore ecosystems damaged by pollutants, has become one of the most rapidly developing fields of restoration ecology (Dobson, Bradshaw & Baker 1997; Cooke & Suski 2008). In the past two decades, this technology has been applied at many sites world-wide. For example, many indigenous or exogenous micro-organisms have been used for degradation of organic pollutants in contaminated environments (Bragg et al. 1994; Cea et al. 2010); various plant species have been widely employed to revegetate mine wastelands (Tropek et al. 2010; Yang et al. 2010), to
establish wetlands for treating municipal wastewater or acid mine drainage (Yang et al. 2006; Chandra & Yadava 2010), and to reduce heavy-metal contamination in agricultural soils (Li et al. 2009). However, a major factor limiting the wider application of this technology involves its relatively low efficiency of contaminant removal (Cooke & Suski 2008).

There is growing evidence that biodiversity generally has positive effects on ecosystem functioning and services (Cardinale et al. 2006; Cadotte, Cardinale & Oakley 2008). Most previous studies have shown that increasing species richness may lead to increased productivity (Tilman et al. 2001), enhanced resistance to environmental perturbations (Mulder, Uliassi & Doak 2001) and decreased likelihood of invasion of exotic species (Kennedy et al. 2002). These advances in understanding of the relationships between biodiversity and ecosystem functions have spurred strong interest in developing diverse ecosystems for enhanced uptake of nutrients from soil and water (Scherer-Lorenzen et al. 2003; Bracken & Stachowicz 2006). There are surprisingly few studies, however, that look at how these advances can be potentially applied to increase the efficiencies of constructed ecosystems for bioremediation of sites contaminated by toxic chemicals.

In a recent study (Li et al. 2010), we showed that increasing species richness can lead to an increase in biomass production of the algal communities growing in substrates contaminated by cadmium (Cd), a highly toxic chemical. More recently, Cardinale (2011) further found that both nitrogen uptake rates and biomass yields of algal communities growing in heterogeneous streams are a linear function of species richness. These findings, taken together with results from previously published studies (see Cardinale et al. 2006; and the references therein), raise a possibility that more diverse ecosystems would be more efficient in removing toxic chemicals (such as Cd) from the environment than would ones with fewer species. To test this possibility, we examined the effects of species richness on Cd removal efficiencies of the algal microcosms, mainly based on the experiment that has been described previously by Li et al. (2010). Specifically, here we provide clear evidence that species richness could have positive effects on Cd removal efficiencies of the algal microcosms, by means of collecting new data on Cd removals of the algal microcosms and performing in-depth analysis of these data as well as those on algal biomass yields that were presented previously (Li et al. 2010). We also show that these positive effects are largely ascribed to the enhanced biomass yields of the polycultures, which are mainly driven by the positive effects of Cd-tolerant species on Cd-sensitive species.

Materials and methods

EXPERIMENTAL DESIGN

We used 10 unicellular green algal species [ Ankistrodesmus falcatus (Af), Chlamydomonas eugametos (Ce), Chlamydomonas moewusi (Cm), Chlamydomonas reinhardtii (Cr), Chlorella pyrenoidosa (Cp), Scenedesmus dimorphus (Sd), Scenedesmus obliquus (So), Scenedesmus quadricauda (Sq), Selenastrum capricornutum (Sc) and Staurastrum polymorphum (Sp)] to construct aquatic microcosms and created experimental units comprising four algal species richness levels (1, 2, 4 and 8). Monocultures of each of the 10 species in our species pool were replicated five times, and for each of the other three richness levels, there were 20 replicates that were assembled by non-repetitive random selection from the species pool. We then crossed the 110 algal communities with three types of growth substrates: Bold’s Basal Medium (BBM) without Cd (control), BBM supplemented with 6 mg CdCl₂ L⁻¹ (moderate Cd pollution) and BBM supplemented with 12 mg CdCl₂ L⁻¹ (severe Cd pollution). This design yielded a total of 330 microcosms. Every microcosm was initially inoculated with the same total number of algal cells (10⁷ cells L⁻¹). We terminated the experiment at the seventh week, when the biomass yields of most algal species had reached a steady state. For more in-depth analysis of the potential roles of different algal species in affecting the ecological functions of these microcosms, we have also further divided the 10 focal species into two functional groups according to their Cd tolerance index (TI): a Cd-tolerant functional group (Af, Cm, Cp and Cr; TI > 100%) and a Cd-sensitive functional group (Ce, Sc, Sd, So, Sq and Sp; TI < 100%). For a full and detailed description of the experiment, see Li et al. (2010).

MEASUREMENT OF BIOMASS YIELD AND CADMIUM CONCENTRATIONS

At the end of the seventh week, we removed 20 mL of medium from each microcosm to determine the biomass yield of each algal species growing in different types of substrates with varying Cd concentrations by cell counting on a hemacytometer (for more details see Li et al. 2010). The remainder of this 20 mL medium was stored at 4 °C until further use.

For the determination of Cd concentration in growth substrate of each Cd-polluted microcosm, the preserved medium mentioned earlier was filtered with quantitative filter paper and then injected into an inductively coupled plasma optical emission spectrometer (Optima 2100 PV; Perkin Elmer Inc., Waltham, MA, USA).

DATA ANALYSIS

The Cd removal of each algal microcosm was calculated by subtracting the Cd concentration in a growth substrate at the end of the seventh week from the initial concentration of Cd in that substrate. We used simple linear regressions to determine the effects of species richness and biomass yield on Cd removal efficiencies of the algal microcosms. According to the method proposed by Loreau (1998) for the determination of over-yielding, we calculated the over Cd removal (Dmax) of each algal polyculture as follows:

$$D_{\text{max}} = \frac{O_Y - \text{Max}(M)}{\text{Max}(M)}$$

where $O_Y$ is the observed Cd removal of a given polyculture, and $\text{Max}(M)$ is the Cd removal by the most efficient monoculture of its component species. Over Cd removal occurs when $D_{\text{max}} > 0$ (Loreau 1998). Simple linear regression was also used to examine the relationship between species richness and the over Cd removal of all the algal polycultures.

After identifying the polycultures with $D_{\text{max}} > 0$, we first examined the relationship between the biomass yields of these polycultures and the magnitudes of the over Cd removal observed in them by performing simple linear regression, to reach a better understanding

of the specific mechanisms underlying the positive $D_{\text{max}}$. We then assessed the relative performance of individual algal species in contributing to the biomass yields of these polycultures, by calculating the proportional deviation ($D_i$) of the biomass yield for each of the 10 focal species with the following equation:

$$D_i = \frac{O_i - E_i}{E_i}$$

where $O_i$ is the observed biomass yield of species $i$ in a given polyculture, and $E_i$ is its expected biomass yield in that polyculture calculated based on its biomass yield in monoculture (Loreau 1998). The average $D_i$ value of each species was tested against zero using a one-sample $t$-test. On this basis, a given algal species was identified as an ‘over-yielding species’ when its $D_i > 0$ (Loreau 1998). Finally, we used a simple linear regression model to examine the effects of biomass yield of individual algal species on the magnitudes of the over Cd removal observed in these polycultures. Statistical analyses were conducted using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA).

Results

This paper focuses on the results for the algal microcosms supplemented with 6 mg CdCl$_2$ L$^{-1}$, given that similar results were obtained for those supplemented with 12 mg CdCl$_2$ L$^{-1}$. Full details of the results for 12 mg CdCl$_2$ L$^{-1}$ are provided in Figs S1–S4 (Supporting information).

Effects of species richness and biomass yields on over Cd removal efficiencies of the algal microcosms

As shown in Fig. 1a, Cd removals by the algal microcosms were positively ($r = 0.30$, $P = 0.001$) correlated with species richness. In monoculture, the most efficient species was $Cm$, which was also the species most tolerant to Cd (see Li et al. 2010 for details) and was able to remove nearly 50% of the Cd in the growth substrate (data not shown). Compared with $Cm$, our most diverse algal polycultures (eight-species) showed a lower Cd removal efficiency (36%), which was marginally significantly ($P = 0.1$) higher that the average (34%) of the four Cd-tolerant algal species in monoculture. However, there were some polycultures that were able to remove Cd to a greater extent than the average of $Cm$ in monoculture ($P < 0.05$, Fig. 1a). On the other hand, there was a significantly positive relationship ($r = 0.25$, $P < 0.01$) between biomass yields and Cd removals by the algal microcosms (Fig. 1b).

Effects of species richness and biomass yields on over Cd removal of the algal polycultures

In all, 27 algal polycultures were able to remove greater amounts of Cd than their most efficient component species ($D_{\text{max}} > 0$, Fig. 2a), representing 45% of the total algal polycultures established in this study. The magnitudes of $D_{\text{max}}$ in all of the algal polycultures were found to be only weakly

![Fig. 1](image-url) Effects of species richness (a) and biomass yield (b) on Cd removal efficiencies of the algal microcosms (6 mg CdCl$_2$ L$^{-1}$). Each data point represents a measurement from one microcosm. The horizontal dashed line and the grey shaded area on the top panel show means ± SE (95% confidence intervals) of the Cd removed by the monocultures of the most efficient species *Chlamydomonas moewusii* (*Cm*). The solid line shown in each panel is the regression line ($r$, Pearson’s correlation coefficient; $P$, P-value).

$(r = 0.13$, $P = 0.045$) correlated with species richness (Fig. 2a). For those polycultures with $D_{\text{max}} > 0$, however, the magnitudes of $D_{\text{max}}$ showed a relatively strong correlation with $(r = 0.41$, $P = 0.033$) the biomass yields of them (Fig. 2b).

Performances of individual species and their effects on over Cd removal of the algal polycultures with $D_{\text{max}} > 0$

In the algal polycultures with $D_{\text{max}} > 0$, three Cd-sensitive species (i.e. *Ce*, *Sd* and *So*) and one Cd-tolerant species (i.e. *Cr*) on average achieved biomass yields higher than expected from their monocultures ($D_i > 0$, $P < 0.05$; Fig. 3), whereas the opposite ($D_i < 0$, $P < 0.05$) was found for two Cd-sensitive species (i.e. *Sq* and *Sc*) and another Cd-tolerant species (i.e. *Af*). On the other hand, the magnitudes of $D_{\text{max}}$ in these polycultures were found to be positively correlated with the biomass yields of a Cd-sensitive species (i.e. *Sd*, $P < 0.05$; Fig. 4), but were not affected by all other species ($P > 0.05$; Fig. S5, Supporting information).
In this study, it was found that Cd removal efficiencies of the monocultures of the 10 algal species significantly increased with the increasing Cd tolerance (as represented by TI) of these monocultures \( (r = 0.80, P = 0.006; \text{detailed data not shown}). \) This result was largely consistent with conventional wisdom (Dobson, Bradshaw & Baker 1997; Gavrilescu 2004). More importantly, we showed that species richness had a positive effect on Cd removal efficiencies of the algal microcosms (Fig. 1a). In theory, the observed positive biodiversity effect could be further partitioned into a ‘selection effect’ and a ‘complementarity effect’ (Loreau & Hector 2001). However, there are no suitable methods currently available to determine Cd removal by each component algal species growing in a polyculture (Lin et al. 2011), which makes it difficult to obtain a direct answer to the question: What was the main driver of the observed positive effect of species diversity on Cd removal efficiencies of the algal microcosms?

Alternatively, we tried to obtain some indirect but explicit answers to this important question by examining the relationships between Cd removal efficiencies and biomass yields of the algal microcosms, because several previous studies have shown that species diversity always achieves a positive effect on an ecosystem function by increasing the biomass yield of that ecosystem (Bracken & Stachowicz 2006; Cardinale 2011). Our analyses showed that Cd removals by the algal microcosms significantly increased with their biomass yields (Fig. 1b), while the amount of Cd removed by per unit algal biomass was not significantly \( (P = 0.21) \) affected by species richness of the microcosms (data not shown). These results, in combination with our previous finding that algal biomass yields of these Cd-polluted microcosms significantly increased with the species richness (see Fig. 1 in Li et al. 2010), indicated that the enhancement of Cd removal efficiencies of the algal microcosms because of the increase in species richness was actually achieved through the enhanced biomass yields in the

**Discussion**

In this study, it was found that Cd removal efficiencies of the monocultures of the 10 algal species significantly increased with the increasing Cd tolerance (as represented by TI) of these monocultures \( (r = 0.80, P = 0.006; \text{detailed data not shown}). \) This result was largely consistent with conventional wisdom (Dobson, Bradshaw & Baker 1997; Gavrilescu 2004). More importantly, we showed that species richness had a positive effect on Cd removal efficiencies of the algal microcosms (Fig. 1a). In theory, the observed positive biodiversity effect could be further partitioned into a ‘selection effect’ and a ‘complementarity effect’ (Loreau & Hector 2001). However, there are no suitable methods currently available to determine Cd removal by each component algal species growing in a polyculture (Lin et al. 2011), which makes it difficult to obtain a direct answer to the question: What was the main driver of the observed positive effect of species diversity on Cd removal efficiencies of the algal microcosms?

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polycultures. On this basis, it might be reasonable to further speculate that the complementarity effect – or more exactly the facilitation provided by Cd-tolerant species was the main driver of the observed positive effect of species richness on Cd removal efficiencies of the algal microcosms, considering that our previous study has shown that the positive relationships between biomass yields of the Cd-polluted algal microcosms and the species richness were largely ascribed to the facilitation provided by Cd-tolerant species (see Li et al. 2010 for more details).

To confirm such speculation, we first identified the polycultures that were able to remove more Cd from the growth substrates than their most efficient component species in monoculture (over Cd removal > 0, $D_{\text{max}} > 0$ for short, Fig. 2a; Loreau 1998) and then we found that these polycultures made up 45% of the total polycultures established in our experiment. The results indicated that these polycultures with $D_{\text{max}} > 0$ were sufficiently significant to warrant extended analyses, given that an up-to-date review of the literature concerning the functional role of producer diversity in ecosystems has revealed that there is currently little evidence to suggest that polycultures can out-perform their most efficient or productive species in monocultures (Cardinale et al. 2011). Intriguingly, we further found that there was a significant positive relationship between the biomass yields of these polycultures and the magnitudes of over Cd removal recorded in them (Fig. 2b). This result, on one hand, lent strong support to the notion that algal species richness achieved a positive effect on Cd removal efficiencies of the microcosms essentially by enhancing biomass yields of the polycultures. On the other hand, it alone did not show whether the observed positive effect of species diversity on Cd removal efficiencies of the algal microcosms was mainly driven by the facilitation provided by Cd-tolerant species, unless we can also demonstrate that the enhanced biomass yields of these polycultures was largely ascribed to the facilitation provided by Cd-tolerant species.

Theoretically, the increases in biomass yield observed in those polycultures with $D_{\text{max}} > 0$ (Fig. 2b) may be driven by a positive selection effect, niche partitioning, facilitation among species or any combination of these three (Loreau & Hector 2001). However, by using $D_{i}$ as a measurement for assessing the relative performances of the 10 algal species in contributing to biomass yields of the polycultures (Loreau 1998), we denied the possibility that a positive selection effect was one of the major causes of the increased biomass yields of the polycultures with $D_{\text{max}} > 0$. If a positive selection effect was predominant in these polycultures, it would require that the majority of the over-yielding species in the polycultures should be Cd-tolerant species ($D_{i} > 0$; Loreau & Hector 2001; Michalet et al. 2006), which was not occurring (Fig. 3). We also excluded the possibility that niche partitioning was one of the major drivers of the increased biomass yields of the polycultures with $D_{\text{max}} > 0$, because homogeneous microcosms were constructed in our experiment, which would greatly limit the opportunities for niche complementarity among the algal species (Li et al. 2010; Cardinale 2011). Based upon our analysis above, we could thus reach a conclusion that the enhanced biomass yields of the polycultures with $D_{\text{max}} > 0$ was largely driven by the facilitation among species. In addition, we also found that the Cd removals by some polycultures were significantly higher than that of the monoculture of the most efficient algal species in this study (Fig. 1), which was strong evidence of facilitation (Hooper & Dukes 2004).

We also further concluded that the facilitation was provided by Cd-tolerant species, because a growing body of literature has demonstrated that facilitative interactions between plant species may occur when environmental harshness is ameliorated by the facilitator species (Brooker & Callaghan 1998; Callaway et al. 2002; Butterfield 2009) and in these Cd-polluted polycultures only Cd-tolerant species had potential to be facilitator species (Brooker & Callaghan 1998; Michalet et al. 2006). Indeed, there were several lines of evidence to support the notion that facilitation occurring in our experiment was provided by Cd-tolerant species. First, it was found that a Cd-tolerant species (i.e. $Af$) yielded lower biomasses than expected ($D_{i} < 0$, Fig. 3), which conforms with conventional wisdom that Cd-tolerant species exposed to Cd pollution tend to allocate more energy to resist the toxicant, leading to reduced energy available for growth and then a consequent lower biomass yield (Baker & Brooks 1989; Gavrilcescu 2004). Furthermore, we also found that the magnitudes of over Cd removal of the polycultures with $D_{\text{max}} > 0$ were positively related to the biomass yields of a Cd-sensitive species (i.e. $Sc$; Fig. 4) but were not affected by those of the four Cd-tolerant species (Fig. S5a–d, Supporting information). A reasonable explanation for this was that the growth of the Cd-sensitive species was promoted when Cd-tolerant species were successful in reducing Cd contents in the substrates to lower levels and then the increased biomasses were employed to absorb more Cd from the substrates (Baker & Brooks 1989; Gavrilcescu 2004).

Collectively, our results provide clear evidence that species diversity may exert positive effects on Cd removal efficiencies of the algal microcosms through facilitative processes among species. More importantly, this study hints strongly that restoration managers may be able to establish some diverse algal polycultures containing both Cd-tolerant species and Cd-sensitive species, which may out-perform their most efficient component species in removing Cd from the environment. These findings are of significance: although the most diverse (eight-species) polycultures did not achieve a higher Cd removal efficiency than the monoculture of the most efficient species in this study, in this era of rapid species loss it would be prudent to employ diverse polycultures rather than risk the loss of individual monocultures. We are also facing a period of unprecedented environmental change (United Nations Environment Programme 2007) and diverse ecosystems tend to be more environmentally stable than ones with fewer species (Tilman, Reich & Knops 2006). However, it should be noted that the Cd-sensitive algal species (i.e. $Sc$; Fig. 4) that performed better than expected and consequently made positive contributions to the enhanced Cd removal efficiencies of the polycultures with $D_{\text{max}} > 0$ is a high-biomass-yielding species ($> 100 \mu g \text{L}^{-1}$). Our results allow us to make a more specific recommendation for the establishment of efficient and stable polycultures.
for removing toxic chemicals (such as Cd) from the environment: the polycultures should contain both toxicant-tolerant species and high-biomass-yielding toxicant-sensitive species. Nevertheless, this study suggests that biodiversity may be an important but previously under-emphasized component of constructed ecosystems for bioremediation of contaminated sites.

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Effects of species richness (a) and biomass yield (b) on overyielding of individual algal species [as represented by proportional deviation (D)] in the polycultures with D_max > 0 (12 mg CdCl₂ L⁻¹). Fig. S4.

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Effects of species richness (a) and biomass yield (b) on Cd removal efficiencies of the algal microcosms (12 mg CdCl₂ L⁻¹). Fig. S2. Effects of species richness (a) and biomass yield (b) on over Cd-removal of the algal microcosms (12 mg CdCl₂ L⁻¹).

Fig. S3. The relative performances of individual algal species [as represented by proportional deviation (D)] in the polycultures with D_max > 0 (12 mg CdCl₂ L⁻¹).

Fig. S4. Effects of biomass yields of individual algal species on the magnitudes of the over Cd-removal observed in the polycultures with D_max > 0 (12 mg CdCl₂ L⁻¹).
Fig. S5. Effects of biomass yields of individual algal species (except Sd) on the magnitudes of the over Cd-removal observed in the poly
cultures with $D_{\text{max}} > 0$ (6 mg CdCl$_2$ L$^{-1}$).

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