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REGULAR ARTICLE

Competition alters plant species response to nickel and zinc

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Abstract Phytoextraction can be a cost-efficient method for the remediation of contaminated soils. Using species mixtures instead of monocultures might improve this procedure. In a species mixture, an effect of heavy metals on the species' performance can be modified by the presence of a co-occuring species. We hypothesised that (a) a co-occuring species can change the effect of heavy metals on a target species, and (b) heavy metal application may modifiy the competitive behaviour between the plants. We investigated these mechanisms in a greenhouse experiment using three species to serve as a model system (*Carex flava*, *Centaurea angustifolia* and *Salix caprea*). The species were established in pots of monocultures and mixtures, which were exposed to increasing concentrations of Ni and Zn, ranging from 0 to 2,500 mg/kg. Increased heavy metal application reduced the species' relative growth rate (RGR); the RGR reduction being generally correlated with Ni and Zn concentrations in plant tissue. *S. caprea* was an exception in that it showed considerable Zn uptake but only moderate growth reduction. In two out of six cases, competitors significantly modified the influence of heavy metals on a target species. The interaction can be explained by an increased uptake of Zn by one species (in this case *S. caprea*) that reduced the negative heavy metal effect on a target species (*C. flava*). In two further cases, increasing heavy metal application also altered competitive effects between the species. The mechanisms demonstrated in this experiment could be of relevance for the phytoextraction of heavy metals. The total uptake of metals might be maximised in specific mixtures, making phytoextraction more efficient.

Keywords Accumulation and tolerance · *Carex flava* · *Centaurea angustifolia* · Heavy metals · Phytoextraction · *Salix caprea*

Introduction

Phytoextraction is increasingly recognised as a cost-efficient, alternative method to physico-chemical technologies used for the remediation of soils contaminated with heavy metals (Meagher 2000; Pilon-Smits 2005; Raskin and Ensley 2000; Suresh and Ravishankar 2004). However, the use of phytoextraction on a larger scale is still in the development phase with recent studies focusing on understanding the underlying mechanisms to improve the efficiency of the procedure (Cobbett 2003; Pilon-Smits and Pilon 2002). Attempts to enhance metal uptake into plants include the use of transgenic plants (Pilon et al. 2003; Uchida et al. 2005) or endophytic bacteria (Newman and Reynolds 2005).

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Phytoextraction is generally carried out with monocultures of plant species. We suggest that remediation might be improved by using species mixtures instead of monocultures. When plants grow in mixtures, their total production is often enhanced by the use of different niches (McKane et al. 2002; Roscher et al. 2005). This generally leads to an enhanced uptake of resources. Moreover, effects of competition and facilitation can modify the growth of a particular neighbour species (Aerts 1999; Coomes and Grubb 2000; Kikvidze et al. 2001). Applied to accumulators on contaminated soils, it is hypothesised that the effects of heavy metals on a species' performance may be modified by the presence of an interspecific competitor; the effect can be enhancing or attenuating. In case of a metal tolerant (hyper-) accumulator, a reduced heavy metal effect on a co-occurring species can be expected. In contrast, species that exclude metals might have negative competition effects on co-occurring species in metal-rich soils. Mitigating effects might be of relevance in phytoextraction in that a pair of species (or several species) can enhance the remediation effect by growing together more vigorously than alone.

So far, the effects of heavy metals on plants were generally investigated on individual species. These studies were focused on the response to metal toxicity (Athar and Ahmad 2002; Briat and Lebrun 1999), the degree of heavy metal uptake by an accumulator (Baker and Brooks 1989), or the potential of individual accumulator species for the phytoremediation of contaminated soils (Citterio et al. 2003; Pulford et al. 2002; Robinson et al. 1998). Studies using species mixtures to evaluate heavy metal effects on interactions among species are rare. Frérot et al. (2006) searched for optimal species mixtures to limit the movement of pollutants out of a contaminated area. In some cases, they found different heavy metal concentrations in plants dependent on a co-occurring species.

Ni and Zn are two common heavy metals in surface soils. Zn is an essential micronutrient of higher plants (Marschner 1995), whereas Ni may be an essential element at least for some but not for all plant species (Gerendas et al. 1999). Above particular concentration levels, both metals impair plants (Shaw et al. 2004). The suggested toxic concentrations in mature leaf tissue are between 10 and 100 mg/kg for Ni and between 100 and 400 mg/kg for Zn (Kabata-Pendias and Pendias 2001; Marschner 1995). Therefore, soil that is contaminated with high levels of Ni and/or Zn has potentially negative effects on plant growth.

In this paper, we used Ni and Zn to examine interactions between the performance of three species and the influence of heavy metals. We experimentally tested the effects of intra- and interspecies competition under increasing soil concentrations of Ni and Zn. We hypothesised that the change from intra- to interspecific competition can modify the degree of heavy metal influence on plants, the modification being amplifying or mitigating. We also hypothesised that heavy metal concentrations in the soil alter the competitive interactions between the species in a mixture. Specifically, we addressed the following questions:

1. Does the occurrence of a competing species alter the influence of heavy metals on growth of another plant species? If yes, is the heavy metal effect enhanced or reduced by competition, and are there differences among various competing species?
2. Does increasing heavy metal concentration enhance or reduce the competitive strength of various competitors?
3. Is the influence of metals on plant performance correlated with metal concentrations in the plant?

Materials and methods

Plant material

Three common European species were selected to serve as a model system in a greenhouse experiment: *Carex flava* L., *Centaurea angustifolia* Schrank, and *Salix caprea* L. (nomenclature following Lauber and Wagner 2007). These species co-occur in their natural habitats and are adapted to wet soil conditions. This adaptation is important since the experiment was run under permanent wet conditions to minimise side effects caused by varying water supply. The species studied included one species known for the phytoextraction of heavy metals (*Salix*; Pulford et al. 2002) and two further species that were expected to interact with the heavy metal effect on *Salix*. Seeds of these species were sown at different times to achieve, as far as possible, equal seedling biomass at the time of transplantation. Seedlings were transplanted into pots on 27 May 2004 (1.7 l pot volume, 13.5 cm tall). Individuals that died during the first 2 weeks of the experiment were replaced, and occasionally occurring volunteer species were entirely removed.

Experimental design

The heavy metal treatment consisted of three different concentrations of either Ni or Zn (applied as $Ni(OH)_2$ and ZnO respectively). Concentration level I was kept uncontaminated to serve as a control. For levels II and III, 1,000 and 2,500 mg heavy metal per kilogram substrate were applied. The heavy metal application may have resulted in a higher soil pH, being caused by an alkaline effect of $Ni(OH)_2$ and ZnO. However, this effect is assumed to be largely buffered by the organic fraction in the soil substrate (Milne et al. 2003). The control (level I) contained 4.6 mg/kg Ni and 17.3 mg/kg Zn, which were the natural background concentrations of the substratum (means of X-ray fluorescence [XRF]-measurements measured on a Spectrolab X-2000, Spectro Kleve, Germany).

The soil substrate was a 1:1 mixture of quartz sand (1–1.7 mm grain diameter) and topsoil from a fen meadow near Frauenfeld, Switzerland. We used 1.7 l PVC pots without water outlet, but established a drainage layer (3 cm) of pure quartz sand (1–1.7 mm grain diameter) at the bottom of the pots. Both the soil substrate and the drainage layer were treated with the heavy metal application. The water level in the pots was permanently adjusted to 3 cm from the bottom.

The three species were combined pairwise, each species competing either with itself or with one of the others. The six possible species combinations (three monocultures, three mixtures) were established at each of the three levels of Ni and Zn, with a separate control for each metal. Six individuals were planted per pot; their location followed the corners of a hexagon with an interseedling distance of 4.5 cm. In case of the two-species mixtures, the species' individuals were placed alternately. Finally, each treatment–mixture combination was replicated four times in blocks, which added up to 144 pots. The set-up was arranged in a randomised complete block design and run in a greenhouse of the Institute of Integrative Biology of ETH Zurich.

Maintenance and measurements

Nutrients were supplied weekly from 2 to 30 June 2004 with a complete fertiliser (including N: 100 g/l, P_2O_5: 100 g/l, K_2O: 75 g/l, B: 102 mg/l, Cu: 81 mg/l, Fe: 190 mg/l, Mn: 162 mg/l, Mo: 10 mg/l, Zn: 61 mg/l; Wuxal, Maag, Switzerland). The N/P ratio was adjusted to 6 with KNO_3. A total of 52.4 mg N, 8.7 mg P, and 0.012 mg Zn per pot was applied. The associated Zn addition to the control was negligible since Zn background concentration in the soil substrate was three orders of magnitude higher.

Plants were regularly checked for pest infestations. Due to an aphid attack on *C. angustifolia*, two insecticides ("Flux C", Maag, Switzerland and "Capito Stop", Landi AG, Switzerland) were sprayed on 8/9 and 16 June 2004.

Initial shoot biomass was estimated by drying and weighing 30 seedlings per species, randomly selected from among those remaining after transplantation. All plants were harvested on 6/7 July 2004, dried at 95°C to constant weight, and the dry mass of every single individual was determined.

After harvest, each pot was filled with deionised water to determine the soil pH. The pH measurements of the soil water were carried out after 4 days of equilibration. Metal concentration of above-surface biomass was determined by XRF- (Spectrolab X-2000, Spectro Kleve, Germany) and ICP-measurements (CCD Simultaneous ICP-OES Vista MPX from Varian). The four replicates needed to be pooled to get sufficient biomass for the measurements.

Data analysis

The net relative growth rate (RGR) between planting and harvesting was calculated following Connolly and Wayne (1996) and Suter et al. (2007). For the calculation of RGR, the biomass of the three individuals per species and pot were pooled. In monocultures, three individuals were randomly selected. The effects of heavy metal concentration and intra- or interspecific competition on the species' performance were analysed with analysis of variance, the RGR of species being the response variable. Ni or Zn concentration and the presence of a particular species as a competitor were explanatory variables, these were defined as factors. The pH was included as a continuous covariable. Multiple comparisons between pairs of means were carried out with the Tukey test (Zar 1999, p. 210), and a general correlation between a species' RGR and the heavy metal concentration in its dry mass was tested with Spearman's rho r_s (Zar 1999, p. 395). All analyses were performed using the statistical software R (R Development Core Team 2007).

Results

To reveal the competition effect on the species' RGR without metal application, the data were first analysed using only the control treatments. There were highly significant competition effects between the three species: *C. angustifolia* was always the strongest competitor followed by *S. caprea*, while *C. flava* was always the weakest ($P \leq 0.05$, Tukey's multiple comparison test on the controls). Generally, both heavy metals had a negative effect on RGR with increasing concentration irrespective of the target species or the competitor (Figs. 1a–c and 2a–c).

Effect of Ni application

C. angustifolia was more affected by Ni application than *C. flava* and *S. caprea* (Table 1, Fig. 1a–c). The RGR decrease of *C. angustifolia* and – less pronounced – of *C. flava* levelled off with increasing Ni concentration (significant quadratic concentration effect—Table 1). *C. angustifolia* also showed the highest Ni concentration in aboveground biomass (Fig. 1e). Its mean accumulation ratio across competitors on levels II and III was 65% and 38%, respectively (accumulation ratio defined as: heavy metal concentration in dried aboveground biomass [mg/kg]/treatment concentration [mg/kg]). The lowest concentrations of Ni revealed *S. caprea* with a mean ratio of 8% on level II and 4% on level III (Fig. 1f). The RGR of all three species was negatively correlated with the Ni concentration in tissue (*C. flava*: $r_s = -0.920$, $P < 0.001$; *C. angustifolia*: $r_s = -0.9$, $P < 0.01$; *S. caprea*: $r_s = -0.9$, $P < 0.01$).

Different competitors significantly affected the RGR of *C. flava* and *C. angustifolia*, but not of *S. caprea* (Table 1). However, competition modified the Ni effect for *S. caprea* (concentration × competitor interaction—Table 1): The RGR of *S. caprea* only decreased from Ni level I to II when competing with *C. flava* (Fig. 1c), no significant RGR reduction of *S. caprea* was detected when growing in monoculture or together with *C. angustifolia*. This indicates that *C. flava* enhanced the effect of Ni on *S. caprea*. Ni addition modified competition on *C. angustifolia* but only at the highest Ni level; here, *C. angustifolia* was favoured when growing with *S. caprea* compared to the situation in monoculture (Fig. 1b). This indicates that the highest Ni concentration mitigated the competitive effect of *S. caprea* on *C. angustifolia.*

Effect of zinc application

C. angustifolia was the most Zn-sensitive species (Table 2, Fig. 2a–c). While the decrease in RGR of *C. angustifolia* was linear with increasing Zn application, *C. flava* and *S. caprea* suffered mainly at concentration level III (Fig. 2a–c, linear and quadratic concentration effect—Table 2). Zn concentration in biomass was negatively correlated with RGR for all three species (*C. flava*: $r_s = -0.73$, $P < 0.05$; *C. angustifolia*: $r_s = -0.9$, $P < 0.01$; *S. caprea*: $r_s = -0.97$, $P < 0.001$). The tissue concentration was highest for *S. caprea* (Fig. 2d–f). This species revealed a considerable Zn tissue concentration on level II, while its RGR was not significantly reduced. The Zn concentration was more than twice the treatment concentration (mean across competitors: 2,250 mg/kg, which equals an accumulation ratio of 225%). The tissue concentration of *S. caprea* at level III was still higher than the application (ratio of 120%). *C. angustifolia*'s Zn concentration was higher than the application at level II (ratio = 166%), but not at level III (ratio = 91%). The accumulation ratio of *C. flava* was always below application (overall mean = 39%).

With the Zn treatments, the significant main effects of interspecific competition were only revealed by *C. angustifolia* (Table 2). Similar to Ni, competition altered the effect of Zn (concentration × competitor interaction—Table 2). *C. flava*'s RGR was significantly reduced from Zn level II to III when competing with itself or *C. angustifolia*. However, when competing with *S. caprea*, the negative Zn effect on *C. flava* was mitigated, and no further RGR reduction was detected at the highest Zn level (Fig. 2a). In one case, Zn altered the competitive effects: Only at Zn level II, *C. angustifolia* was negatively affected when competing with *S. caprea* compared to the situation in monoculture (Fig. 2b), while such an effect could not be detected for Zn levels I and III.

Effect of species combinations on pH

Species effects on pH were observed in the pots without heavy metal application (Fig. 3). The pH values in pots

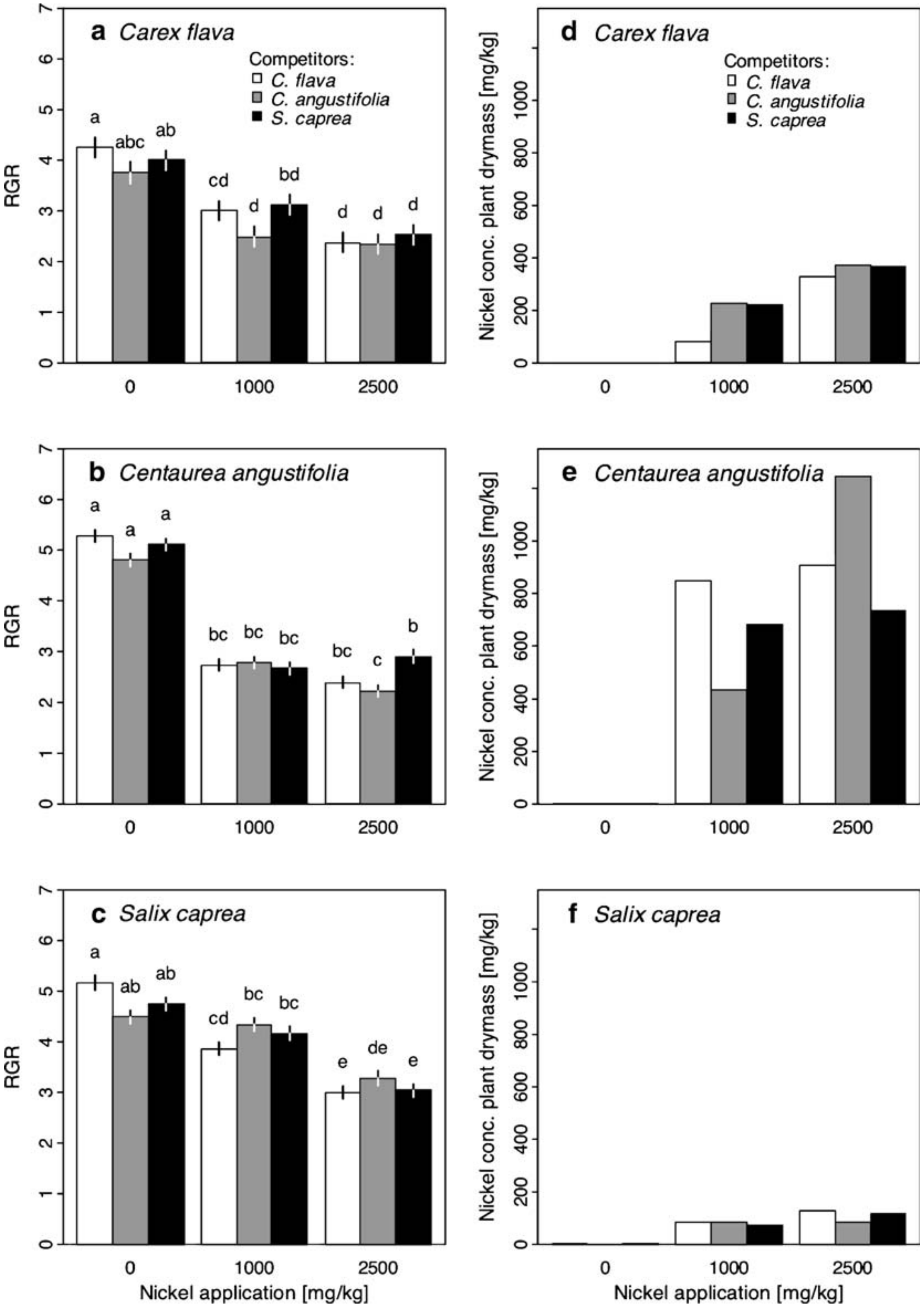


Fig. 1 **a**–**c** Effect of Ni application and competitors on the RGR of three fen species. Data are predicted means±1 SE according to three ANOVAs, one per species. *Different letters* indicate a difference at $P \leq 0.05$ (Tukey's multiple comparison test). **d**–**f** Ni uptake of the three species depending on Ni application and competitor. Plant material of the four replicates had to be pooled, thus no tests of significance could be performed

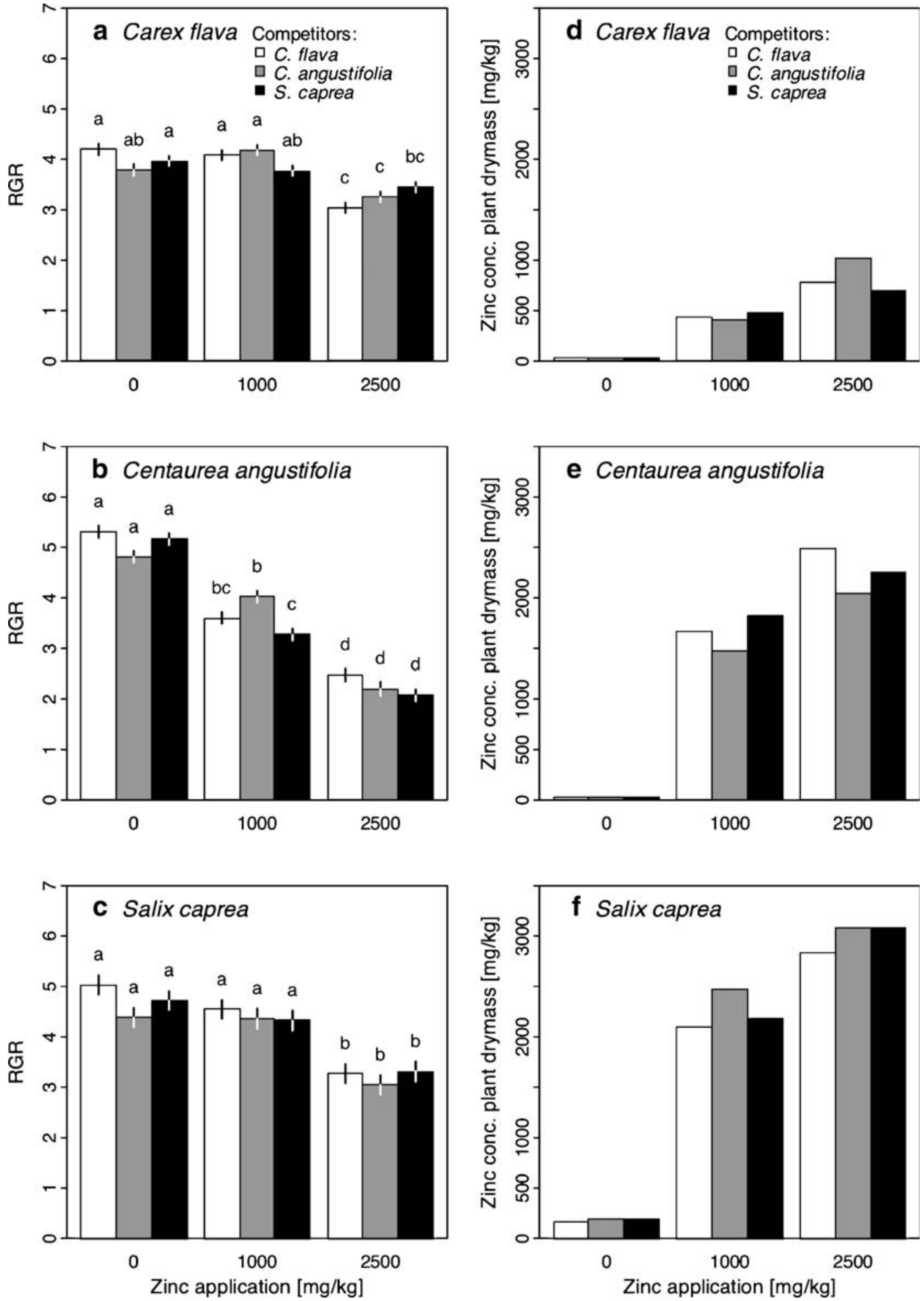


Fig. 2 **a**–**c** Effect of Zn application and competitors on the RGR of three fen species. Data are predicted means±1 SE according to three ANOVAs, one per species. *Different letters* indicate a difference at $P \leq 0.05$ (Tukey's multiple comparison test). **d**–**f** Zn uptake of the three species depending on Zn application and competitor. Plant material of the four replicates had to be pooled, thus no tests of significance could be performed

Table 1 Effects of three levels of Ni concentration (Conc.) and different competitors (Comp.) on the RGR of three fen species (results of three separate ANOVAs)

Source[a]	Target species								
	C. flava			*C. angustifolia*			*S. caprea*		
	df	MS	*F*	df	MS	*F*	df	MS	*F*
Concentration	2	7.985	54.26***	2	23.855	451.10***	2	8.391	132.68***
Linear effect	1	15.065	102.38***	1	39.048	738.40***	1	16.534	261.44***
Quadratic effect	1	0.905	6.15*	1	8.662	163.81***	1	0.248	3.92
Competitor	2	0.533	3.62*	2	0.330	6.25**	2	0.089	1.41
pH	1	0.046	0.31	1	0.0002	0.004	1	0.043	0.67
Conc.×Comp.	4	0.118	0.80	4	0.212	4.01*	4	0.339	5.36**
Residuals	26	0.147		25[b]	0.053		25[b]	0.063	

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

[a] Model evaluation based on the AICc criterion (Burnham and Anderson 2002) revealed, that the block term could always be omitted

[b] One missing value

with monocultures of *C. angustifolia* (predicted mean: 5.12) or *S. caprea* (5.36) were significantly lower compared to those with *C. flava* monocultures (5.64). In pots with heavy metal applications, no significant species effects on soil pH could be detected.

Discussion

General effects of competition and heavy metal application

Without heavy metal application, the competitive potential of the three species declined in the order of *C. angustifolia*, *S. caprea*, and *C. flava*. The superiority of *C. angustifolia* may be explained by allelopathy, since allelopathy in *Centaurea* species has been demonstrated several times (Fletcher and Renney 1963; Ridenour and Callaway 2001). Besides being the strongest competitor and having the highest RGR on the controls, *C. angustifolia* suffered most from Ni and Zn addition. This strong reaction may be related to increased metal availability through soil acidification; *C. angustifolia* lowered the pH in the soil more than the other species (Fig. 3). We also observed the same behaviour in a preliminary experiment (data not shown). Lowering the pH increases the availability of phosphate and other micronutrients (Dakora and

Table 2 Effects of three levels of Zn concentration (Conc.) and different competitors (Comp.) on the RGR of three fen species (results of three separate ANOVAs)

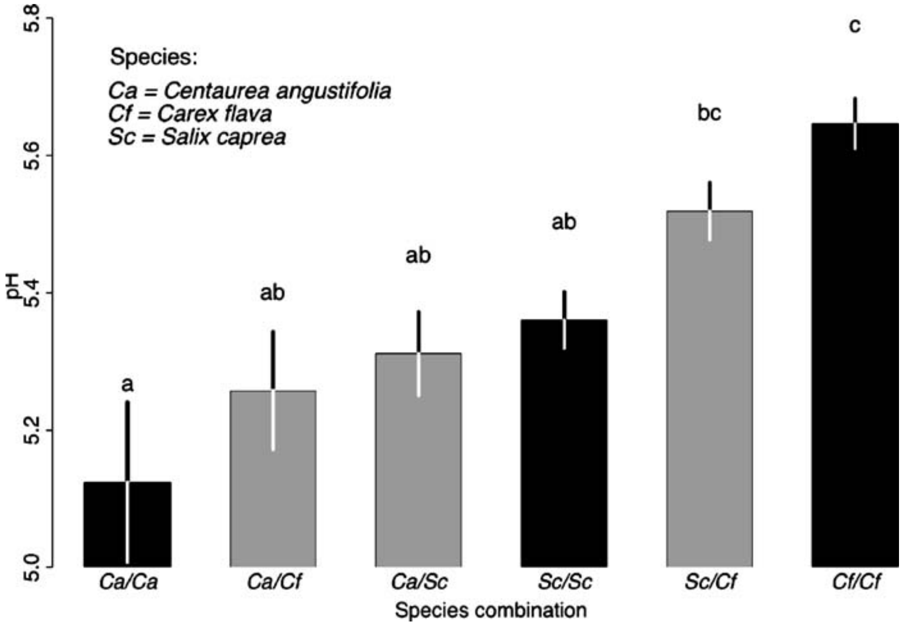
Source[a]	Target species								
	C. flava			*C. angustifolia*			*S. caprea*		
	df	MS	*F*	df	MS	*F*	df	MS	*F*
Concentration	2	2.261	56.21***	2	23.067	412.31***	2	7.556	54.16***
Linear effect	1	3.257	80.98***	1	46.116	824.32***	1	13.494	96.73***
Quadratic effect	1	1.265	31.45***	1	0.017	0.31	1	1.618	11.60**
Competitor	2	0.003	0.08	2	0.408	7.30**	2	0.367	2.63
pH	1	0.064	1.59	1	0.165	2.95	1	0.012	0.09
Conc.×Comp.	4	0.226	5.62**	4	0.367	6.56***	4	0.070	0.50
Residuals	26	0.040		25 [b]	0.056		25[b]	0.140	

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

[a] Model evaluation based on the AICc criterion (Burnham and Anderson 2002) revealed, that the block term could always be omitted

[b] One missing value

Fig. 3 Effects of monocultures (*black bars*) and species combinations (*grey bars*) on pH in pots without heavy metal application. Displayed are means±1 SE. Multiple comparisons were performed with the Tukey test



Phillips 2002), but may also enhance metal-availability in the rhizosphere (Marschener 1998). Both a higher metal availability and enhanced toxicity effects for *C. angustifolia* compared to the other two species are supported by high Ni and Zn tissue concentrations (Figs. 1 and 2).

Although increasing heavy metal application levels generally resulted in decreasing RGRs for all three species, the intensity of the metal influence was not strictly correlated with metal concentration in plants, a statement also made by Baker and Walker (1990). For example, *S. caprea* was able to accumulate Zn, but was only moderately affected by Zn application (Fig. 2c and f). Nissen and Lepp (1997) showed that *S. caprea* (amongst other *Salix* species) accumulated Zn in shoot tissue up to five times the soil concentration without being considerably reduced in growth. Further *Salix* varieties (also including crossings between *Salix* sp.) are known to accumulate considerable amounts of different heavy metals with a good survival rate and biomass production (Pulford et al. 2002).

Interactions between competition and heavy metals

If species such as *Salix* are grown under heavy metal influence in mixtures with others, they will not only accumulate heavy metals, but also competitively interact with each other. A species that accumulates metals, and at the same time is tolerant to metal, can incorporate considerable amounts in its tissues (Pulford and Watson 2003). Such behaviour is likely to influence a co-occurring species. In our experiment, we found significant competitor×concentration interactions in four out of six cases; *S. caprea* was involved in all of them. These general interactions alone (Tables 1 and 2) do not define whether competition altered the heavy metal effect on plants, or *vice versa*, whether the heavy metal effects changed the competitive influence. However, the distinction can clearly be made by the post-hoc tests (Figs. 1 and 2). If, for example, heavy metal application only affects a target species when it is together with one particular interspecific competitor, this specific neighbour must have changed the heavy metal effect. In contrast, an effect of heavy metal on competition can be seen, when there is no difference between intra- and interspecific competition in all but one particular heavy metal application level. In this case, the heavy metal effect on that level must have modified competition.

For both Ni and Zn, one competing species significantly altered the influence of the involved heavy metal on the target species: *C. flava* enhanced the Ni effect on *S. caprea* (Fig. 1c), and *S. caprea* attenuated the negative Zn effect on *C. flava* (Fig. 2a). In the latter case, the mitigating effect can be explained by the strong Zn uptake of *S. caprea*. Due to its high metal accumulation, *S. caprea* might have lowered the availability of Zn for *C. flava* (the target species) and thus mitigated the negative metal effect on the sedge. Positive interactions between plant species have gained increasing attention in recent years (Callaway and Walker 1997; Callaway et al. 2002; Zanini et al. 2006). They are explained by provision of shade, mitigation of disturbance, or protection from herbivores by some species which can enhance the performance of neighbouring species (Callaway et al. 2002). It can therefore be assumed that a high uptake of heavy metals by one species will also positively influence neighbouring species. The first case, where *C. flava* enhanced the Ni effect on *S. caprea*, is more difficult to explain. Such enhancements can occur when one species increases the availability of heavy metals by lowering the pH with excessive root exudates as discussed above for *C. angustifolia*. In our case, however, *C. flava* did not show such behaviour.

We also found two cases (one for each metal) where heavy metal application influenced the competitive behaviour of a species: For instance, medium Zn application (1,000 mg/kg) enhanced competition of *S. caprea* on *C. angustifolia* (Fig. 2b). Metal tolerant species – both accumulators and excluders – are often unaffected by high levels of heavy metals. Kayama et al. (2005) demonstrated that the dry mass of *Picea glehnii* on serpentine soils was almost the same as on brown forest soils. In contrast to the brown forest conditions, the serpentine soil had a high content of Ni, but *P. glehnii* was able to exclude Ni and to maintain low concentrations of the heavy metal. As a result, *P. glehnii* was more tolerant to serpentine soil conditions than the other spruce species investigated. A species such as *P. glehnii* might be equally competitive to a non-tolerant species on unpolluted conditions. However, the competitive behaviour might change under heavy metal influence in that the tolerant species grows more vigorously relative to non-tolerant neighbours. In our case, increased Zn application moderately affected *S. caprea*, and this strong performance of *S. caprea* with heavy metal application could explain its increased competition over *C. angustifolia*. At level III, performance of *S. caprea* was also impaired by Zn (Fig. 2b and c).

Relevance for phytoextraction

This study aimed at identifying mechanisms between heavy metal influence on species and their competitive interactions. Some mechanisms demonstrated here could be useful for the phytoextraction of heavy metals from contaminated soils. We suggest three cases, where the use of mixtures instead of monocultures could be important and which should receive further investigation:

First, a hyperaccumulator could be grown together with a species that reduces negative heavy metal effects on neighbours. The accumulators' performance would be increased (or less reduced) and it would be able to extract higher amounts of metal from the soil. Mitigation occurred between *S. caprea* and *C. flava* under Zn influence in our experiment. Mitigating effects also include direct facilitation between species, and facilitation has been found to be important for community dynamics in various habitats (Bellingham et al. 2001; Callaway et al. 2002; Franks 2003). Recently, Frérot et al. (2006) demonstrated higher biomass in mixtures than in monocultures on a highly polluted site. The effect was attributed to facilitation between the nitrogen fixing *Anthyllis vulneraria* and the co-occurring grasses; however, increased total uptake of heavy metals was not detected.

Second, one could search for a combination of two hyperaccumulating species with strong overyielding. Overyielding is present when the species' performance in mixtures exceeds the performance expected from the respective monocultures (Kirwan et al. 2007; Roscher et al. 2005). Overyielding can generally be explained by different niche use (Fox 2003; Tilman 1999). If overyielding is present or even enhanced by species interactions under increased heavy metal concentration, the extracted amount of heavy metals should be greater than growing each species alone, provided the uptake rates remain high.

Third, an application for the phytoextraction of heavy metals could be tested with the combined growth of a hyperaccumulating species with a species that lowers the pH in the rhizosphere and thus increases the availability of metals (Dakora and Phillips 2002; Gahoonia 1993). In the present experiment, such a pair is represented by *S. caprea* and *C. angustifolia* under Zn influence. Both species showed considerable high Zn tissue concentrations, but Zn concentrations in *S. caprea* tended to be higher when growing with *C. angustifolia* than on its own. This behaviour is likely to be caused by the influence of *C. angustifolia* on the soil pH. Exudates other than organic acids released by a plant species may also influence the heavy metal uptake of a neighbour species (Buschmann et al. 2006). This can occur directly by influencing soil conditions relevant for the uptake of heavy metals or indirectly by ameliorating soil conditions which enhances the productivity. For example, release of phosphatase increases phosphate availability.

This study showed that interactions take place between the species' performance and heavy metal influence when grown in mixtures and that these interactions could be relevant for phytoextraction. Further research should focus on suitable combinations of hyperaccumulating plant species that maximise the total uptake of heavy metals by plants and thus make the phytoextraction process more efficient.

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