SSD

SCIENCE FOR A SUSTAINABLE DEVELOPMENT

Biodiversity impacts of highly invasive alien plants: mechanisms, enhancing factors and risk assessment

"Alien Impact"

I. Nijs, M.Verlinden, P. Meerts, N. Dassonville,
S. Domken, L. Triest, I. Stiers, G. Mahy, L. Saad,
A.-L. Jacquemart, V. Cawoy

ENERGY
TRANSPORT AND MOBILITY
AGRO-FOOD
HEALTH AND ENVIRONMENT
CLIMATE
BIODIVERSITY
ATMOSPHERE AND TERRESTRIAL AND MARINE ECOSYSTEMS
TRANSVERSAL ACTIONS

SCIENCE FOR A SUSTAINABLE DEVELOPMENT
(SSD)

Biodiversity

FINAL REPORT

Biodiversity impacts of highly invasive alien plants: mechanisms, enhancing factors and risk assessment

"Alien Impact"

SD/BD/01

Promotors
Ivan Nijs
University of Antwerp (UA)
Department of Biology
Research Group Plant and Vegetation Ecology

Pierre Meerts
Free University of Brussels (ULB)
Laboratory of Plant Genetics and Ecology

Ludwig Triest
Free University of Brussels (VUB)
Department of Biology
Research Group Plant Science and Nature Management

Grégory Mahy
Gembloux Agro Bio Tech (GxABT)
Laboratory of Ecology

Anne-Laure Jacquemart
Catholic University of Louvain (UCL)
Departement of Biology
Unit of Ecology and Biogeography

Authors
Ivan Nijs, Maya Verlinden (UA)
Pierre Meerts, Nicolas Dassonville, Sylvie Domken (ULB)
Ludwig Triest, Iris Stiers (VUB)
Grégory Mahy, Layla Saad (GxABT)
Anne-Laure Jacquemart, Valérie Cawoy (UCL)

D/2012/1191/14
Published in 2012 by the Belgian Science Policy
Avenue Louise 231
Louizalaan 231
B-1050 Brussels
Belgium
Tel: +32 (0)2 238 34 11 – Fax: +32 (0)2 230 59 12
http://www.belspo.be

Contact person: Aline Van der Werf
+32 (0)2 238 36 71

I. Nijs, M.Verlinden, P. Meerts, N. Dassonville, S. Domken, L. Triest, I. Stiers, G. Mahy, L. Saad, A.-L. Jacquemart, V. Cawoy - Final Report. Brussels : ***Biodiversity impacts of highly invasive alien plants: mechanisms, enhancing factors and risk assessment "Alien Impact"*** Belgian Science Policy 2012 –xx pp. (Research Programme Science for a Sustainable Development)

TABLE OF CONTENT

SUMMARY

Context

Information on the impacts of alien invasive plant species on ecosystems is scarce, but critical to protecting biodiversity and ecosystem functions in a world with increasing trade, travel and transport. Impacts seem to vary with spatial scale (from microsite to landscape) and ecological complexity (individual, population, community, ecosystem), and both direct and indirect underlying mechanisms have been suggested. Information is especially scarce on the subtle effects of invasive plants that cannot readily be observed (e.g. on other trophic groups), yet this is highly needed to estimate the full threat to biodiversity. Forecasting the impact of Belgian alien invasive plants faces the challenge that detailed studies (by necessity limited to few species/sites) are needed to disentangle the coupling of response mechanisms at different ecological scales, whereas general trends can only be derived from assessments with simple measures over a large scale (many sites).

Objectives

The ALIEN IMPACT project aimed to provide a first integrated study of patterns and mechanisms of impact by alien invasive species in Belgium. It considered multiple, highly invasive plant species (HIPS), and combined large-scale screening of invader impact (to characterize patterns) with highly mechanistic studies at fixed sites to characterize impact pathways. Both terrestrial and freshwater ecosystems were studied. The main objectives were: (1) To identify the **patterns of HIPS impact** on the diversity of native plant communities, by characterizing communities that experience greatest impact and characterizing target native species, both in aquatic and terrestrial ecosystems. (2) To identify **mechanisms of HIPS impact** on native plants, both direct and indirect via pollinators or soil modification. (3) To estimate the **impacts at other trophic levels** by investigating whether HIPS impact on native plant diversity is associated with diversity loss or changes in community structure in other trophic groups, notably soil fauna and macro-invertebrates in water and sediment. (4) To analyse **factors that may modify HIPS impacts** on native plant species in the future.

Does eutrophication or climate warming alter the competitive ability of HIPS?

Conclusions

Patterns of HIPS impact on the diversity of native plant communities

We examined the effect of seven highly invasive plant species (4 terrestrial plants: *Fallopia* spp., *Senecio inaequidens*, *Impatiens glandulifera* and *Solidago gigantea* and 3 aquatic plants: *Hydrocotyle ranunculoides, Ludwigia grandiflora and Myriophyllum aquaticum)* on native species richness, abundance and composition. In terrestrial systems, especially *Fallopia* spp. and *S. gigantea* exhibited a strong impact on native species richness, starting already at low densities. *Impatiens glandulifera* and *Senecio inaequidens*, on the other hand, had less impact, except for the latter species at high density. In aquatic ecosystems all HIPS induced strong declines in native species richness, mainly affecting native submerged and floating species because these occupy the same position in the water column as the invaders. Across terrestrial and aquatic systems, impact generally correlated well with density of the invasive species.

Mechanisms of HIPS impact on native plants

A study on indirect impacts by HIPS via pollinators in terrestrial and aquatic systems investigated whether HIPS (*Fallopia* spp., *S. inaequidens*, *I. glandulifera*, *S. gigantea*, *L. grandiflora*) affect reproductive success of native plant species and whether those impacts are mediated by modification of pollinator services. The results show that both terrestrial and aquatic HIPS are highly attractive to a large number of native pollinators and are well integrated in native plant-pollinator networks. There was however no strong evidence of invader impact on native pollinator services. Weak facilitation effects were detected for *L. grandiflora* and *I. glandulifera*. The reproductive success of the native species was not affected by the studied HIPS.

Two experiments examined the underlying mechanisms of HIPS impact on native terrestrial plants via soil modification. One study investigated the impact of *F. japonica* on nitrogen cycling and another experiment studied impacts of *S. gigantea* on phosphorus. The results show that both these invasive species influence specific processes in the cycling of nutrients in the plant-soil system, resulting in alterations in topsoil nutrient pools. *F. japonica* produces recalcitrant litter that immobilizes N, while the species has an efficient resorption in belowground organs and greater internal recycling of N than native plants. This results in a decreased N availability to native species. *S. gigantea* increases the available P pool, most likely due to a pH decrease and its fine root dynamics. In conclusion, manipulation of key limiting resources appears to play a prominent role in the competitive superiority of both species.

In an experiment on the impact of HIPS on competing native species via modification of soil properties, the hypothesis of a positive feedback of *F. japonica* on its own competitive success was tested but rejected. No significant difference was observed between plant performance in invaded and uninvaded soils, suggesting there is no memory effect of past invasion by this species. However, both in invaded and uninvaded soil, the native competitor *C. arvense* grew better in pure culture in the absence of charcoal (charcoal immobilizes soluble organic compounds, like allelopathic substances, in the soil) while it grew better in mixed culture in soil amended with charcoal. This indicates that the competitive superiority of *F. japonica* is probably partially due to allelopathic properties.

Impacts at other trophic levels

In terrestrial systems, impacts on soil fauna were examined for *F. japonica*, *S. gigantea*, *S. inaequidens* and *I. glandulifera*. Soil fauna density most strongly declined under *F. japonica*, while under *I. glandulifera* the total number of individuals increased. Observed impacts could be explained by altered microclimatic conditions, by changes in litter chemical composition and by decreased native plant diversity. In aquatic systems we investigated whether the invasive *H. ranunculoides*, *L. grandiflora* and *M. aquaticum* modify the invertebrate, phytoplankton and zooplankton abundance and diversity. There was no clear support for impacts of HIPS on overall species diversity. All three HIPS negatively affected invertebrate and zooplankton abundance, which could be explained by reduced space, sunlight and oxygen exchange in invaded ponds. Phytoplankton density increased in highly invaded ponds, which may be caused by the entrapment capacity of the invasive species.

Factors that may modify HIPS impact on native plant species

We investigated the effect of soil eutrophication on the competitive balance between terrestrial native and invasive alien plant species (*F. japonica*, *S. gigantea* and *S. inaequidens*) and the effect of water eutrophication on the competition between the invasive *Lagarosiphon major* and the native *Ceratophyllum demersum*. For both terrestrial and aquatic invasive species, the results do not support the hypothesis that eutrophication consistently shifts the competitive balance in favour of the invasive species. In terrestrial communities, the trends varied with the studied species. The competitive superiority of the invasive species decreased with fertility in the case of *F. japonica* while it increased for *S. inaequidens*. Eutrophication did not affect the competitive ability of *S. gigantea*. Nutrient inputs into soils thus favour specific HIPS but suppress others. In the aquatic communities, the invasive *L. major* had a better performance than its native competitor, and eutrophication did not modify this balance.

Simulated climate warming had different effects on the competitive interactions between terrestrial invasive and native species depending on the studied species pair and on the experimental climate conditions. In an experiment where all plants received optimal water supply, climate warming reduced the invader dominance of *S. inaequidens*, but stimulated the suppressed invader *S. gigantea*. These responses could mostly be traced to root specific nitrogen uptake capacity. In an experiment where warming was associated with soil drought, climate change tended to increase the dominance of *S. inaequidens*, in agreement with the warmer and drier climate in its native range and with its significantly enhanced photosynthetic rates observed in the experiment. The competitive balance of the other two studied HIPS (*S. gigantea* and *F. japonica*) and their native competitors was not influenced by warming. The observed warming effects on the competitive interactions in these two experiments could for many cases be explained by the intrinsic warming responses of the species.

Contribution of the project in a context of scientific support to a sustainable development policy

Overall, our results support that HIPS do more to ecosystems than merely suppress native competitors. A wide range of HIPS impact exist, both in terrestrial and aquatic systems, and a number of these are severe.

HIPS severely endanger species diversity both in terrestrial and aquatic communities, but differences exist which could be useful to guide control. In terrestrial systems, even low densities of *Fallopia* spp. and *S. gigantea* exhibited a strong impact on native species diversity, so for those species, management at the very beginning of invasion is necessary to prevent impact on native plant communities. The presence of HIPS in nature reserves seems to be rather linked to common habitats, characterized by ruderal species. This points to the importance of avoiding disturbance in sites of high biological value to limit nascent foci of invasion. In aquatic systems, regarding negative impacts on diversity, one group of native species was particularly sensitive: submerged species. Ponds with those growth forms may require priority for control.

The HIPS in this study did not have clear negative impacts on the reproductive success of selected native species. However, our results cannot be generalized to all native and invasive species. Recent literature shows that pollinator-mediated impacts of invasive species on natives are species-specific and identifying invasion-sensitive native plant species is crucial to improve conservation strategies.

The soil compartment plays a key role regarding mechanisms of HIPS impact on terrestrial systems. *F. japonica* had a negative impact on organic matter cycling and the data suggest that this impact may last after *F. japonica* is removed, possibly requiring topsoil removal to restore invaded sites after control. *S. gigantea* decreases soil pH and enhances P availability. For this species, liming could be considered as a control measure.

Effects of HIPS can strongly proliferate to other trophic levels in both aquatic and terrestrial ecosystems. The strongest impact was found for *F. japonica*. The impact of this species was greater in open habitat than in closed vegetation, suggesting that open habitat should be given priority in control of this species.

A final word of warning concerns human-induced factors that may modify HIPS impact. We specifically refer to *S. inaequidens*, which currently exhibits more modest impacts than the other HIPS that we examined. Our climate change experiments suggest that this may change in the future as climate warming tends to increase the competitive superiority of this species. At the same time, *S. inaequidens* reacted well to eutrophication. These characteristics warrant close surveillance of the future evolution of this species.

Keywords: biological invasions, highly invasive plant species, terrestrial ecosystems, aquatic ecosystems, biodiversity

1. INTRODUCTION

While anthropogenic global change has made some species decline, others have thrived and proliferated, sometimes with dramatic impacts on biodiversity. Such species are referred to as 'invasive'. Most recent authoritative reviews define alien invasive species or taxa as (1) being an alien (species, subspecies or lower taxon, introduced outside its natural past or present distribution), (2) reproducing and increasing its range in its new environment (Richardson et al. 2000; Pysek et al. 2004). The introduction and spread of non-native species has become a global ecological and conservation crisis as invasive organisms are increasingly altering terrestrial and aquatic communities worldwide (Byers 2002, Levine et al. 2003, Ehrenfeld 2006, Mason & French 2007, Lau 2008). In this context, assessing the effects of invasive nonindigeneous species on native species and ecosystems is now one of the world's most urgent conservation issues (Byers 2002). To date, the impacts of invasive plants are not well known. Impacts seem to vary with spatial scale (from microsite to landscape) and ecological complexity (individual, population, community, ecosystem), and both direct and indirect underlying mechanisms have been suggested. Information is especially scarce on the subtle effects of invasive plants that cannot readily be observed (e.g. on other trophic groups), yet this is highly needed to estimate the full threat to biodiversity. Developing effective prevention strategies and management solutions, requires that impacts are characterized beyond the anecdotic level of (mostly single-invader) case studies. To what extent do impacts follow general patterns across alien species and invaded communities? Which environmental factors mitigate or aggravate impact?

The desire to respond effectively has prompted governments to call for improved strategies for reducing nonindigeneous species' impacts at national, regional and local levels. To achieve this goal, the scientific basis for decision-making on biological invasions needs to be improved, in line with the priorities of international research agendas. Understanding and quantifying impacts of biological invasions also fits in several prioritary fields of international conventions to which Belgium is committed (e.g. Convention on Biological Diversity).

The aim of the project was to provide a first integrated study of patterns and mechanisms of impact by alien invasive species in Belgium. It considered different spatial scales and multiple levels of ecological organisation. The project considered both terrestrial and fresh water ecosystems. Its central aim was impact on biodiversity. We focused on impact on native autotrophics, but also on soil and water fauna, as well as on how eutrophication (soil and water) and climate warming (only terrestrial) modify impact. Both direct (via competition) and indirect (via pollination, soil modification, allelopathy) mechanisms of impact were studied. The project concentrated on highly invasive plant species in Belgium. Forecasting the impact of Belgian alien invasive plants faces the challenge that detailed studies (by necessity limited to few species/sites) are needed to disentangle the coupling of response mechanisms at different ecological scales, whereas general trends can only be derived from assessments with simple measures over a large scale (many sites). The aim of the current project was to reconcile these conflicting prerequisites in a single study.

2. METHODOLOGY AND RESULTS

A. PATTERNS OF IMPACT

We examined the effects of seven highly invasive plant species that were introduced in Belgium: 4 terrestrial plants (*Fallopia* spp., *S. inaequidens*, *I. glandulifera* and *S. gigantea*) and 3 aquatic plants introduced through the aquarium trade (*H. ranunculoides, L. grandiflora and M. aquaticum)*. For the two groups of species the aim of the study was to assess to what degree invasive species cover directly influences the assemblage of native plants. Specifically, we address the following questions: 1) Is there an effect of HIPS on native species richness, abundance and composition? 2) If so, is this effect similar for each invasive species? 3) What is the direction and magnitude of the impact?

Materials and methods

In total, 42 terrestrial sites and 32 water bodies on 22 sites in Belgium were sampled for the impact study on native vegetation. The studied sites represent a gradient of percent cover or density of one of the invasive species studied. A particular focus was set on sites of high biological value both for terrestrial and aquatic plants (e.g. nature reserves and Natura 2000 sites). To determine the impact of HIPS in terrestrial systems, 12 plots (1 m^2) were sampled in each site: 6 in invaded and 6 in uninvaded vegetation (total number of plots: 502). Invaded plots were sampled along gradients of HIPS density. Within each plot, the cover of other plant species was recorded at the time of HIPS flowering. To determine the impact HIPS in aquatic systems two approaches were used, at a plot and pond levels. For the plot approach, when available, three invasion categories (plots, 4 m^2) were distinguished: (A) plots in an uninvaded water body, (B) semi-invaded plots in an invaded water body, and (C) heavily invaded plots (cover > 75%) in an invaded water body. B and C plots were in the same water body (invaded ponds) while A plots were in a separate water body (uninvaded ponds) but in close vicinity. The cover of native and invasive species was estimated for each of the 114 plots. For the pond approach we recorded native plant cover along a gradient of percent invasive species cover. Cover estimates were made at the level of three growth forms, namely submerged, free-floating/floating-leaved and emergent.

Results

Impact on native aquatic plant species

Uninvaded plots (A) harboured in total 28, 20 and 20 native species in close vicinity of infestation with *H. ranunculoides*, *L. grandiflora* and *M. aquaticum* respectively. These values compare to 17, 24 and 18 species respectively in semi-invaded plots (B) and to 9, 12 and 9 species in heavily invaded plots (C). Recorded species included 12 submerged species, 10 floating-leaved and free floating species (e.g. Lemnids) and 29 emergent species.
Uninvaded A plots of *H. ranunculoides* harboured on average 5.20 ± 1.11 species compared to 1.60 ± 0.22 species in heavily invaded C plots. This difference in species number was statistically significant (Kruskall-Wallis: χ^2_2 = 8.80, $P < 0.01$, Figure 1). For *L. grandiflora* the three invasion categories differed significantly in species richness (ANOVA: $F_{2,38} = 23.30$, $P < 0.001$, Figure 1). For *M. aquaticum* uninvaded A plots had significantly higher plant species richness (4.00 ± 0.70) than heavily invaded C plots (1.71 ± 0.45) (ANOVA: $F_{2,35}$ = 4.21, $P < 0.05$, Figure 1). On average, the impact of *H. ranunuloides* and *L. grandiflora* was stronger (decrease of about 70%) compared to a 57% decrease for *M. aquaticum* from uninvaded A plots to heavily invaded C plots.

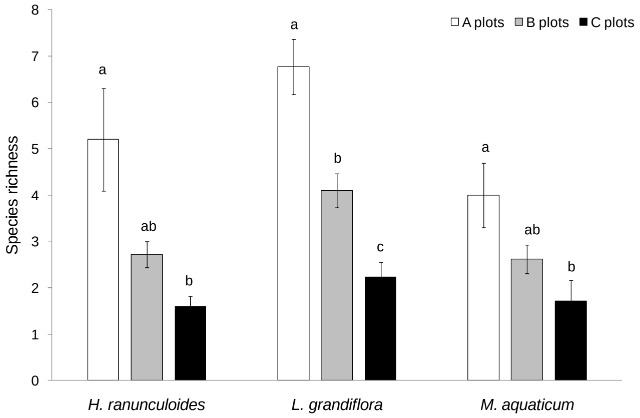


Figure 1: Impact of colonization of *H. ranunculoides* (n = 38), *L. grandiflora* (n = 41) and *M. aquaticum* (n = 35) on native plant species richness by invasion categories (A plots = uninvaded, B plots = semi-invaded, C plots = heavily invaded). Given are means ± SE; different letters indicate significant differences amongst invasion categories within each invasive vegetation type ($P < 0.05$)

Kruskall-Wallis ANOVA by ranks was undertaken for 14 species in total. Of these, only four species exhibited significant differences in abundance among the different invasion categories, only for *H. ranunculoides* and *L. grandiflora* invaded plots (Table I).

Table I Significant differences in native species abundance amongst *H. ranunculoides* and *L. grandiflora* uninvaded (A) plots, semi-invaded (B) plots and heavily invaded (C) plots. Different letters indicate significant differences (with a ranked greater than b)

Invasive species	Native species	Invasion category			χ^2	P
		A	B	C		
H. ranunculoides	Submerged					
	Ceratophyllum demersum	a	a	b	13.600	0.001
	Floating					
	Lemna minor	a	b	ab	14.048	0.002
L. grandiflora	Submerged					
	Ceratophyllum demersum	a	b	b	27.897	< 0.001
	Emergent					
	Alisma plantago-aquatica	a	ab	b	7.565	0.023
	Lycopus europaeus	a	ab	b	7.095	0.029

A strong negative relationship was found between invasive species cover and submerged species cover and between invasive species cover and floating (leaved) species cover among all ponds and among invaded ponds only (Table II). The effect of invasive species cover on emergent vegetation was only marginally significant among all ponds. Native submerged vegetation was present in 80% of the uninvaded ponds, compared to 41% of the invaded ponds.

Table II Gamma correlation coefficients between invasive species cover and the three growth forms (submerged species cover, floating (leaved) species cover, emergent species cover) among all ponds (n = 32) and among invaded ponds only (n = 22). * denote statistical significance

Growth form		Γ	P
Submerged species cover	All ponds	-0.56	< 0.001*
	Invaded ponds	-0.43	0.042*
Floating (leaved) species cover	All ponds	-0.39	0.014*
	Invaded ponds	-0.63	0.001*
Emergent species cover	All ponds	-0.30	0.060
	Invaded ponds	-0.03	0.899

In almost every invaded and uninvaded pond, native floating (leaved) and emergent vegetation was present but the abundance declined when invasion increased.

In ponds with an invasive species cover of > 50% there was a decline of submerged species cover, floating (leaved) species cover and emergent species cover of 94.5%, 68% and 44% respectively, when compared to uninvaded ponds. When the invasive species cover reached 75% there was no submerged vegetation left.

Impact on native terrestrial plant species

Despite the fact that 67% of the selected sites were nature reserves and/or sites of high biological value, a large majority of the invaded habitats were characterized by common plant species and none of the invaded plant communities was classified as Natura 2000 habitat. No native species with patrimonial value (red list) were found in uninvaded plots.

A total of 54, 97, 70 and 65 species were recorded in invaded vegetation respectively for *Fallopia spp*. (N=10), *S. inaequidens* (N=10), *I. glandulifera* (N=11) and *S. gigantea* (N=11), compared to 110, 104, 97 and 122 species respectively in adjacent uninvaded vegetation. At the plot level ($1m^2$), a significant decrease of species richness was observed in invaded plots compared to uninvaded plots (nested two ways ANOVA) (Figure 2) for *Fallopia* spp (uninvaded : 7.2 ± 3.7 (mean ± SD), invaded : 3.2 ± 2.6 , P<0,001); *I. glandulifera* (uninvaded : 6.9 ± 2.9, invaded : 3.2 ± 2.6 , P<0,001), *S. gigantea* (uninvaded : 7.6 ± 4.6, invaded : 4.6 ± 2.3 , P<0,001). In all cases, a significant interaction was found between the invasion effect at the plot level and the site (details not shown), indicating that the extent of decrease varied from site to site. The mean magnitude of species richness decrease in invaded plots varied considerably among the three species: *Fallopia* spp.: 55%; *S. gigantea*: 33%; *I. glandulifera*: 16%. In contrast, species richness did not differ significantly between uninvaded and invaded plots for *S. inaequidens* (ANOVA, P = 0,455).

For the three HIPS that exhibited a global effect of invasion on species richness, a significant relationship (linear regression) between density (percentage cover) of the HIPS and the number of native species at $1m^2$ scale was found for *Fallopia* spp. and *S. gigantea* but not for *I. glandulifera* (Figure 3). The number of native species was also correlated to density in *S. inaequidens* despite no general pattern of species richness reduction was found in invaded plots compared to uninvaded ones.

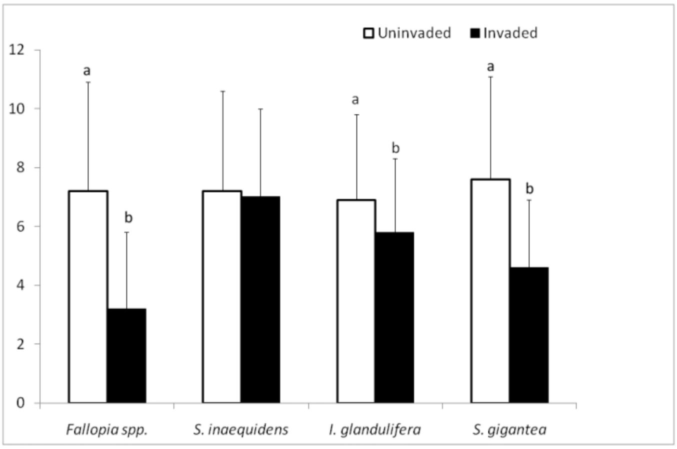


Figure 2: Impact of invasion by *Fallopia spp., S. inaequidens, I. glandulifera* and *S. Gigantean* on native plant species richness at plot scale (1 m²) by invasion categories (uninvaded and invaded). Given are means ± SD; different letters indicate significant differences amongst invasion categories within each invasive vegetation type (ANOVA, $P < 0.05$)

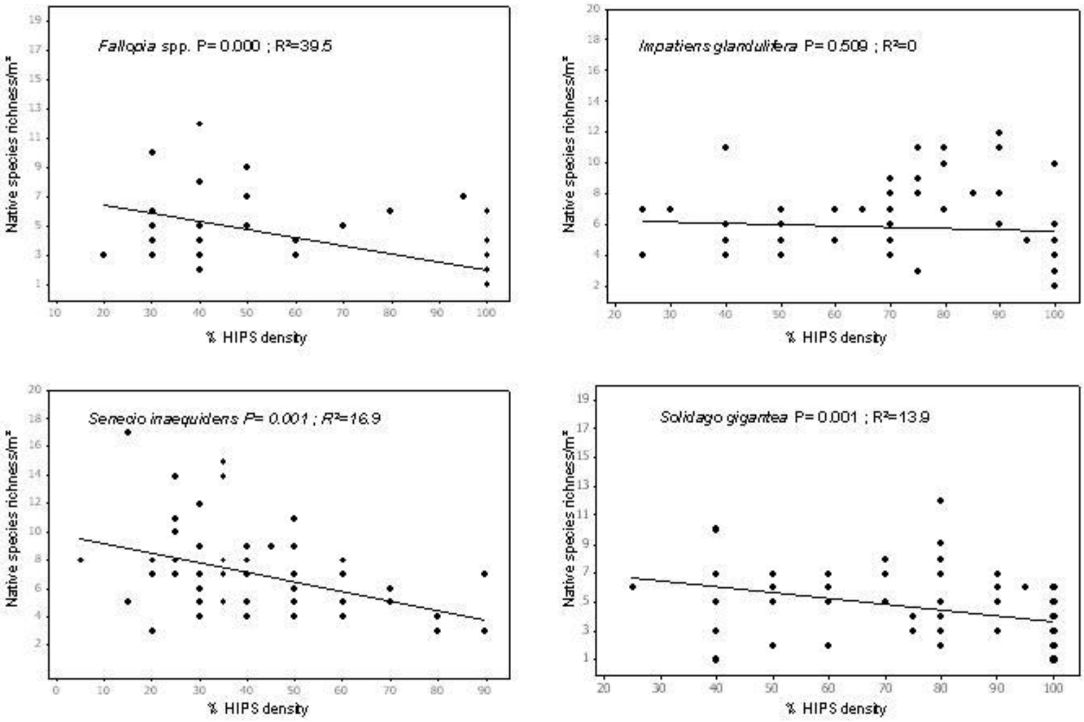


Figure 3: Linear regressions of native plant richness (per m²) vs. HIPS density in invaded plots (% cover) for the four target HIPS.

Correspondance analysis indicated similar plant species composition for vegetation invaded by *Fallopia* spp., *S. gigantea* and *I. glandulifera*, while vegetation invaded by *S. inaequidens* was characterized by a different floristic composition (Figure 4). There was no strong evidence of differences in plant species composition between invaded and uninvaded vegetation for any HIPS. Diagnostic species for invaded and uninvaded vegetations for each HIPS were determined with the IndVal method (Indval 2.0, Dufrene and Legendre 1997). No native species were preferentially associated to the invaded plant communities compared to univaded communities. For each HIPS, the Jaccard index of similarity (JI) was computed independently between pairs of invaded vegetation (JI invaded) and between pairs of uninvaded vegetation (JI uninvaded) at site scale. The ratio JI invaded/JI uninvaded was used as a measure of vegetation homogenization after invasion. A ratio >1 indicates homogenization and <1 differentiation. Homogenization of plant composition was recorded in 44, 42, 53 and 64% of the occurrences (plots) respectively for *Fallopia* spp., *I. glandulifera*, *S. inaequidens* and *S. gigantea* (Figure 5). In the case of *Fallopia* spp., *I. glandulifera*, *S. inaequidens*, invasion did not significantly increase the Jaccard index (p > 0.05), while it did for *S. gigantea* (p = 0.02), meaning that the latter species has a significant homogenizing effect.

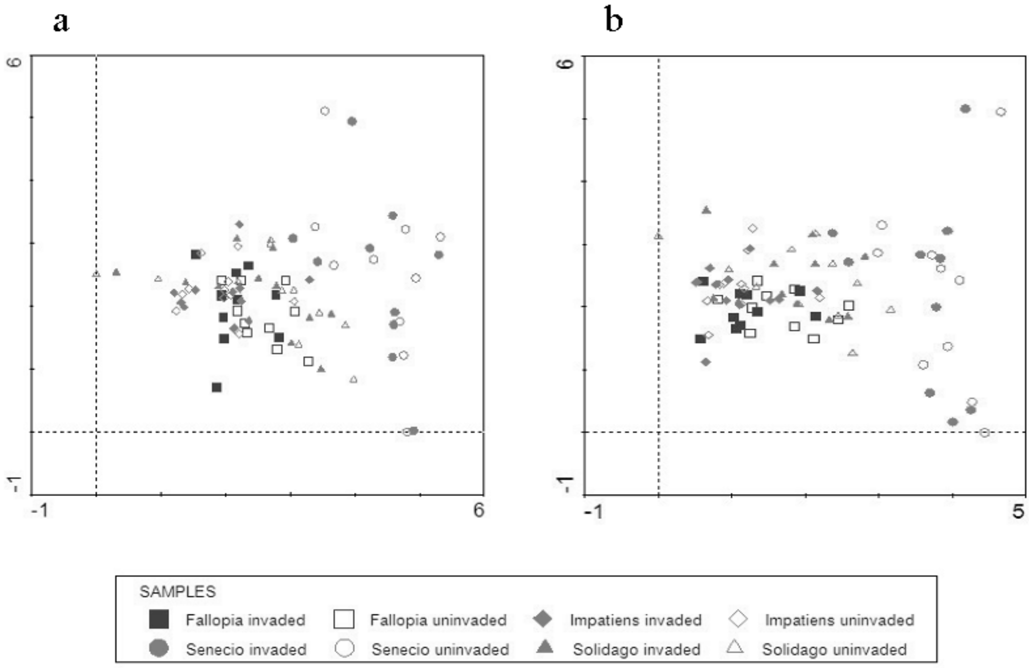


Figure 4: Correspondence analysis ordination graph of plots realised from (**a**) species abundance data (**b**) presence-absence data.

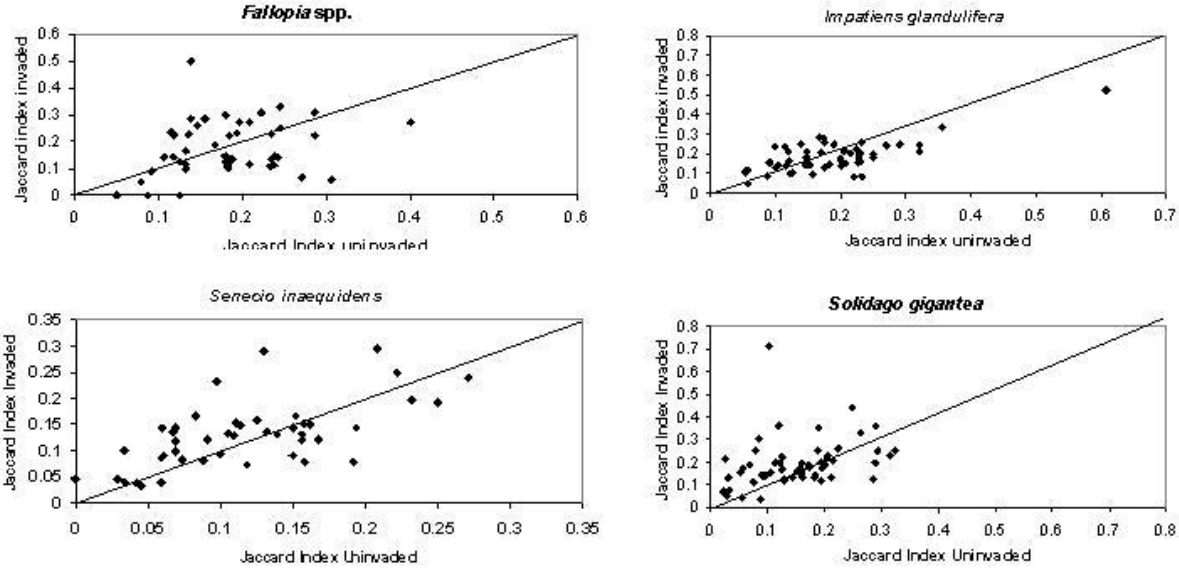


Figure 5: Comparisons of Jaccard similarity index between invaded and univaded vegetations : 20 out of 45, 23 out of 55, 24 out of 45 and 35 out of 55 comparisons between sites invaded respectively by *Fallopia* spp., *I. glandulifera glandulifera*, *S. inaequidens* and *S. gigantea*, and adjacent uninvaded vegetation, occur above the line of isometry (slope = 1, y-intercept = 0), indicating that the invasion by these HIPS tends to increase similarity between plant communities in 42% to 64% of the occurrences.

Discussion

Much attention has been paid to negative effects of alien species on resident communities but studies that quantify community-level effects of a number of invasive plants are scarce. The most comprehensive multi-species study of HIPS impact on native plant communities was only recently delivered by Hejda et al. (2009) by comparing impacts of 13 invasive terrestrial species on native plant richness and community composition. In the present study, we extend the multi-species approach by considering both terrestrial and aquatic species. Our results are largely concordant with the conclusions of Hejda et al. (2009). We clearly demonstrate a strong negative impact of invasive species on native species richness in both terrestrial and aquatic systems. In general, species richness was lower in invaded sites compared to univaded sites and we found a general trend for a negative association between density of the invasive species and native species richness. However, this effect of invasion was largely species-specific, and the severity of impact depends on the identity of particular invading species.

In the aquatic systems, invaders usurp a large amount of space throughout the water column. However, the response of the three different growth forms to the invasion differed considerably. These compositional changes were exhibited mainly by the submerged growth forms and the floating growth forms validating the results of Hussner (2008) who described the displacement of native submerged aquatic species due to the expansion of dense floating mats of *H. ranunculoides* and *M. aquaticum*. Native submerged and floating species occupy the same position in the water column as the invaders and hence competition is expected to be intense (Gopal and Goel 1993). Their rapid growth rates will allow them to capture the available resources more efficiently. Similar impacts of loss of native vegetation have been reported for the invasive *Eichhornia crassipes* in Lake Chivero, Zimbabwe (Brendonck et al. 2003). Before the expansion of water hyacinth in the lake, submerged and rooted floating-leaved macrophytes were common in the shallow parts. Ali et al. (2006) reported the replacement of the originally dominant submerged macrophyte *Najas marina* subsp. *Armata* by the invasive *Myriophyllum spicatum* in Lake Nasser, Egypt. Collectively, these findings highlight the major impacts that aquatic plant invaders have on submerged species. Impacts of *H. ranunculoides*, *L. grandiflora* and *M. aquaticum* invasion amongst individual species were difficult to obtain probably because of a limited number of present native species.

As a general pattern, the invasion of terrestrial HIPS decreases the available area and cover of other plant species, which in turn lowers the plant species richness. However, the magnitude of this impact is species specific. As found in other studies, *Fallopia* spp. and *S. gigantea* impact seriously native species richness (Hulme & Breemer 2006, Hedja et al. 2009). *I. glandulifera* and *S. inaequidens*, on the other hand, exhibited a much lower impact, except for the latter species at high density. It is a clear-cut observation that high densities of the target invasive species reduce native plant richness in terrestrial systems. The impact of an invader is expected to correlate with its own population density, since any biomass (or space or energy) controlled by the invader constitutes resources no longer available to other species (Daehler 2003). However we found that low densities can already induce a substantial impact in the cases of *Fallopia* spp. And *S. gigantea*. For those species management at the very beginning of invasion is necessary to prevent any impact on native plant communities. No systematic effects of invasion were found on plant composition, when comparing invaded and uninvaded stands despite species loss (decrease of species richness). This means that changes were mainly random. This clearly contrasts with the results of Hedja et al. (2009) who found a clear divergence between invaded and uninvaded vegetation. Differences in invaded vegetations may explain this contrast. Most invaded communities were dominated by ruderals. It has been reported that it is resistant ruderal species that increase their representation in invaded communities (Hejda & Pyšek 2006). However, the importance of the impact detected in our study for terrestrial species should be considered on the view that none of the invaded vegetation was classified as high biological value.

B. MECHANISMS OF IMPACT

ELUCIDATING DIRECT IMPACT VIA TRAIT OVERLAP

We investigated if trait overlap between the invasive *S. gigantea* and the native vegetation can explain the outcome of native-invasive competition at microscale. The following questions were addressed: (1) Does *S. gigantea* grow better were it is surrounded by native neighbours with different or with similar traits, in other words, does trait overlap explain invader success? (2) Does invader impact on its native neighbours depend on trait overlap?

Materials and methods

In a site invaded by *S. gigantea*, 21 plots were selected. Each plot consisted of one individual *S. gigantea* plant and its ten closest neighbour species. Plots varied in density and identity of neighbour species. Leaf gas exchange was measured on all species with a portable gas exchange system (LI-6400, Li-cor, NE, USA): light-saturated photosynthetic rate (P_{max}), dark respiration rate (R_d), stomatal conductance (g_s), and transpiration rate (Tr). Water use efficiency (WUE) was calculated as P_{max}/Tr. On the leaf samples used for these leaf gas exchange measurements, specific leaf area (SLA) was calculated as leaf area/dry leaf mass. Finally, all plants were harvested aboveground, dried for 48 h and weighed (B_{above}).

For each of the traits mentioned above (except B_{above}), the dissimilarity between the invader and his native neighbours per microsite was calculated as $|X_{native} – X_{invader}|$, where X_{native} is the average value of trait X of all 10 native plants and $X_{invader}$ is the invader value of trait X. Euclidian distance was used to combine dissimilarities of all traits. Next, we used linear regression to examine the relationship between the dissimilarities of all traits (separately and combined) and the biomass of *S. gigantea* (question (1)) and between the dissimilarities and the average biomass of the native plants (question (2)). All statistics were performed using SPSS 16.0 (SPSS Science, Woking, UK).

Results

Concerning question (1), we hypothesized that a low trait overlap (= large dissimilarity) means little competitive interference which will lead to high productivity of *S. gigantea*. This was found for two of the observed traits, P_{max} and g_s (linear regression, $F_{1,19} = 22.849$, $p < 0.05$; $F_{1,19} = 7.018$, $p < 0.05$ for P_{max} and g_s, respectively, Figure 6). For the other traits, no significant relationship was found between the dissimilarities and the invader biomass, neither for the combination of all traits.

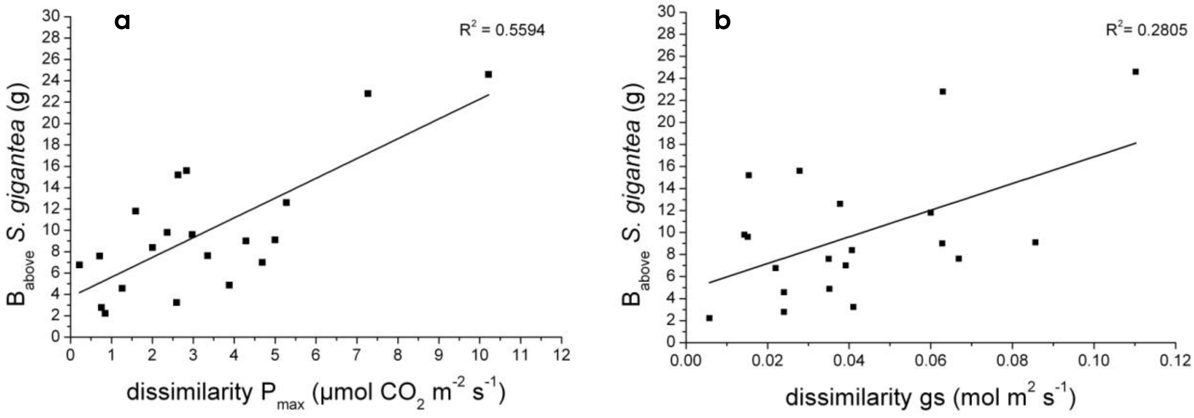


Figure 6: Invader success. Linear regression of aboveground biomass of *S. gigantea* against the dissimilarity in P_{max} (a) and g_s (b) between natives and *S. gigantea*. Means per plot.

Regarding the relationship between trait overlap and invader impact (question 2), little competitive interference as a consequence of low trait overlap may lead to high productivity of the native plants (= low impact of *S. gigantea*). For none of the measured traits (separately or combined) such a relationship was found.

Discussion

Ricciardi & Atkinson (2004) have shown that an invader's impact is determined by the system's evolutionary experience with similar species: high-impact invaders are more likely to belong to genera not already present in the system, suggesting that lack of niche overlap is at the basis of invader success. If invasive plants fill empty niches (cf. species coexistence theory of

Aarssen, 1983), this may explain their success. This is what we hypothesized and was to some extent confirmed by our results. Success of the invader increased when overlap in two of the measured traits (P_{max} and g_s) between the invader and native neighbours became smaller. This did however not involve an increased impact of the invader, which could result from the age of the invader plants. We selected one-year old *S. gigantea* individuals in order to exclude differences in growth that were caused by age differences and not by potential effects of the native neighbours. As a consequence, *S. gigantea* may have been too small to have impact. Also the fact that all native neighbours are not only influenced by the invader but also by other native plants may have precluded an effect of trait overlap on the impact of *S. gigantea*.

INDIRECT IMPACT MEDIATED BY POLLINATORS

The aim of this task was to assess whether selected HIPS (four terrestrial species: *Fallopia* spp., *S. inaequidens I. glandulifera*, *S.gigantea*; one aquatic species: *L. grandiflora*) affect reproductive success of native plant species and whether those impacts are mediated through modification of pollinator services. The general research strategy was to assess pollination interactions between each selected HIPS and a native counterpart. The native counterparts should meet three criteria in relation to the HIPS: 1) co-occurrence in the field, 2) similar visitor guilds and 3) overlapping flowering periods. For three species (*S. inaequidens*, *I. glandulifera*, *L. grandiflora*) a native counterpart was identified on the basis of previous studies or literature data. In contrast, for *Fallopia* spp. and *S. gigantea,* given the lack of information on pollinator guilds and their sharing with native species, a food web approach (sensu Memmot 1999) was applied as a first step to study species interactions in the field. The selection of a native competitor for controlled experiments was performed by crosschecking pollinator guilds and flowering phenology between invasive and native species. Due to constraints in experimental design, no species-species study was realized for *Fallopia* ssp. Methodology and results are presented separately for each case.

FINDING A NATIVE COUNTERPART: FIELD OBSERVATIONS AND PLANT POLLINATOR NETWORKS FOR *FALLOPIA SPP.* AND *S. GIGANTEA*

Material and Methods

In this task we addressed the following questions: 1) do native plants and HIPS share similar phenology? 2) What is the degree of pollinator sharing?
Three study sites encompassing rich plant communities were selected per HIPS (*Fallopia* spp. and *S. gigantea*). In each site, insect visits for all plant species of the community were observed during a fixed time (2 minutes/m²) in 2 x 2 m quadrats along 10 m long permanent transects. The identities of the visiting insects were recorded for each observed plant-insect interaction: plants at the species level and category identification of the insect species (e.g. small Syrphidae). Insects were collected on the same quadrats for further identification. On each site, observations were carried out every two weeks during the HIPS flowering period, adding up to three to four observations per site.

Results

Both HIPS were integrated into the native pollination network (Figure 7). They mainly attracted dipterans (> 80 % of visits performed by flies, hoverflies and other small dipterans). At the site/community level, between 35 and 57% of total insect visits were recorded on *S. gigantea*, and between 35% and 88% on *Fallopia* spp. This correlates with the abundance of HIPS, which provide a high proportion of the overall floral resources in invaded sites.

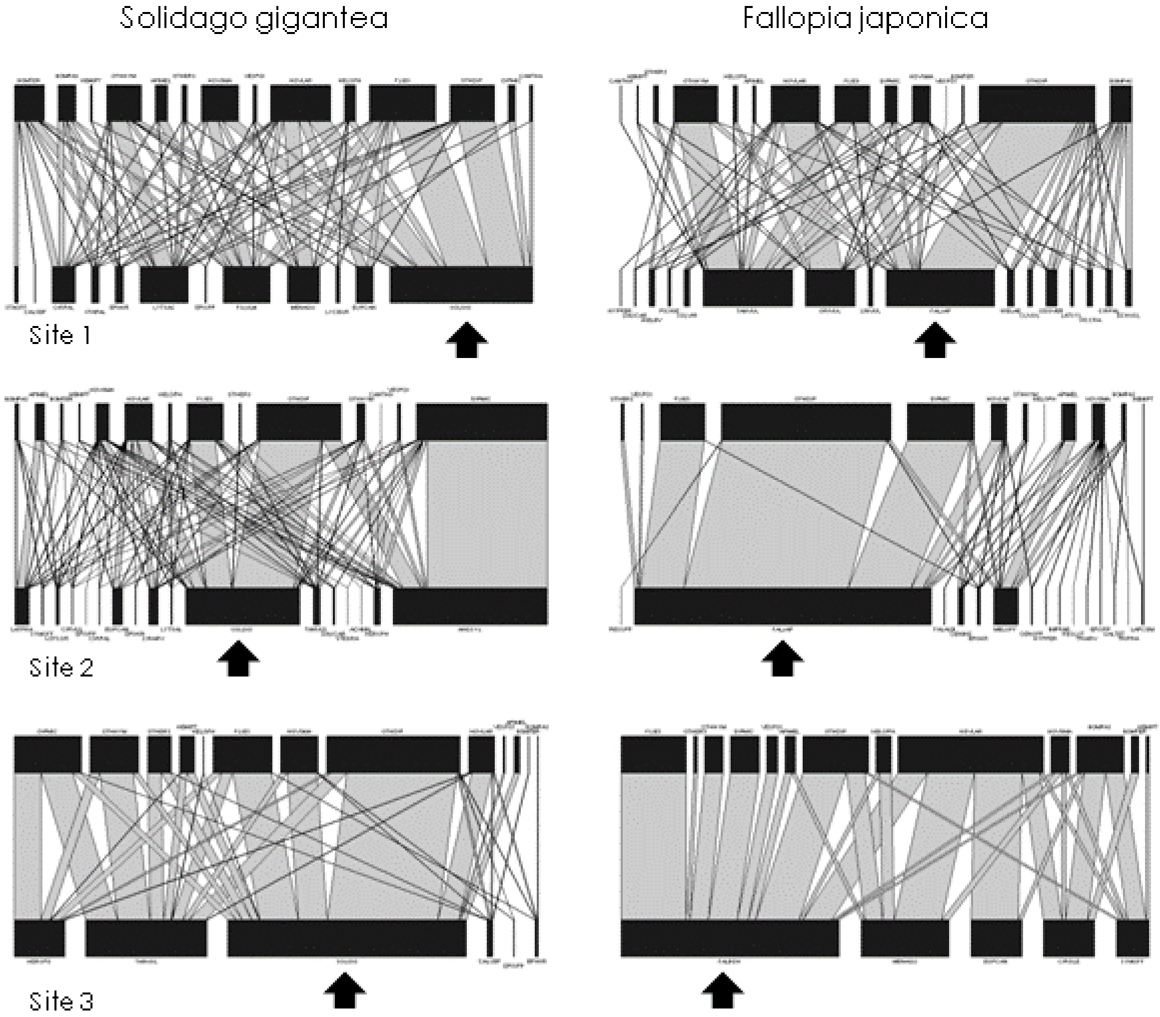


Figure 7: Integration of *S. gigantea* (left) and *Fallopia* spp. (right) in the plant pollinator network of native plant communities at three sites. Interactions were recorded between plant species (below) and insect categories (above). The length of the species bar is proportional to the importance of the species in plant-insects interactions. The width of the grey links indicates the strength of the interaction between a given plant species and a given insect category. Arrows indicate HIPS.

INTERACTIONS IN INVADED SITES BETWEEN *S. GIGANTEA* AND A NATIVE COUNTERPART, *DAUCUS CAROTA*

Material and Methods

We examined the effect of the invasive *S. gigantea* on flower visitation patterns and seed set of the co-flowering native *D. carota*. The native counterpart was selected on the basis of pollination web analysis. We placed and observed flowering *D. carota* plants in three invaded and three non-invaded sites, using the same experimental design. On each site, 18 potted *D. carota* plants were placed, half of them ("cultivated") were previously cultivated in the greenhouse and the other half ("wild") was directly taken from the wild. Both types of potted plants were placed at 0 m, 4 m and 8 m from the invasive species, in three replicates per site (Figure 8).

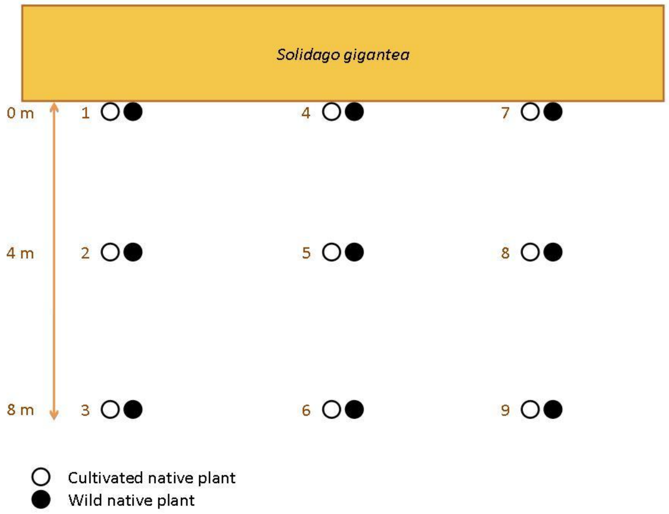


Figure 8: Experimental design involving potted *D. carota* plants placed at different distances from the invasive *S. gigantea*.

Visitation rates were recorded on "wild" plants only, based on 10 minutes observation periods twice a day (am & pm) during 6 days over the period July – August 2010. Visitation rate was defined per plant as the number of insects observed on umbels divided by the number of flowering umbels per plant. Visitation rates were averaged for the 6 days, separately for morning and afternoon observations. In order to compare invaded and uninvaded sites, visitation rates were analysed using a three-way ANOVA with "invasion" (fixed, two levels), "time of the day"(fixed; two levels: am & pm) and "site" (random; nested within invasion; six levels). The factors invasion and time of the day were crossed. A second analysis was performed only for invaded sites, in order to detect an influence of the distance to the invasive population. A three-way ANOVA with the factors "time of the day" (fixed; two levels: am & pm), "distance" (fixed; three levels: 0, 4 and 8 m) and "site" (random; three levels) was used. All the factors were crossed.

At the end of the flowering season, the number of (1) well- developed seeds; (2) aborted seeds and (3) non-fecundated ovules was recorded on two umbels from all plants (wild and cultivated). "Efficient seed set" was defined as ((1)/((1)+(2)+(3)))*100 and averaged per plant. "Pollination rate" was defined as ((1)+(2)/((1)+(2)+(3)))*100 and averaged per plant as well.

Efficient seed sets and pollination rates were analysed using a three-way ANOVA with "invasion" (fixed), "growing conditions"(fixed; two levels) and "site" (random; nested within invasion; six levels). The factors invasion and growing conditions were crossed. Then, a three-way ANOVA with the factors "growing conditions" (fixed; two levels), "distance" (fixed) and "site" (random; three levels) was used on the data from invaded sites only. All the factors were crossed. In the case of a significant effect, Tukey simultaneous pairwise comparisons were used.

Results

Visitation rates, seed set and pollination rate were not significantly different between invaded and non-invaded sites, and neither the factor site nor any interactions were significant. However, a significant difference in visitation rate was found between morning and afternoon observations, with a higher visitation rate in the morning (P= 0.021). In invaded sites, there was an influence of the distance to the invasive population on visitation rates (P= 0.019). Pairwise comparisons revealed that the closest plants to the invasive populations had lower visitation rates (Figure 9). In all sites, efficient seed set and pollination rate were significantly higher for wild plants as compared to those cultivated in the glasshouse (respectively P= 0.012 and P= 0.023). Seed set and pollination rate were not influenced by the distance to the invasive population.

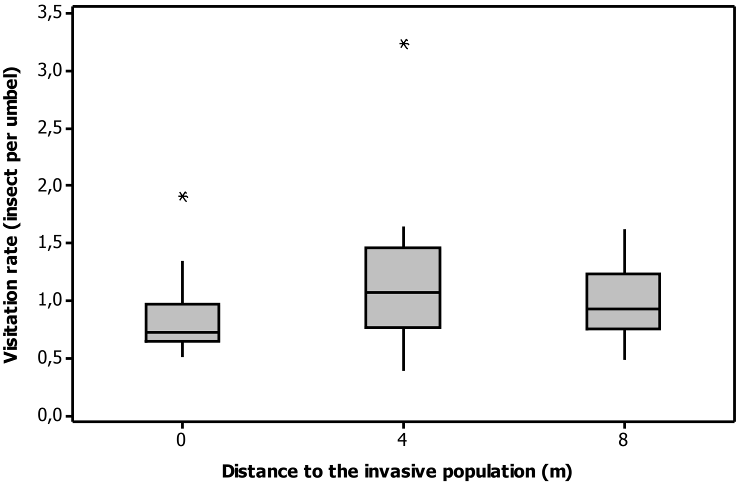


Figure 9: Boxplots of the visitation rates on *D. carota* plants observed during 12 ten-minutes periods during summer 2009, in relation to the distance to *S. gigantea* populations.

INTERACTIONS IN EXPERIMENTAL FIELDS BETWEEN *S. INAEQUIDENS – I. GLANDULIFERA* AND NATIVE COUNTERPARTS

Material and methods

For *I. glandulifera*, we selected two native competitors based on HIPS pollinator observations in the field and on literature data: *Epilobium angustifolium* and *Aconitum napellus* subsp. *lusitanicum*. The latter was chosen as a second case study to explore the potential threat of HIPS to native species of patrimonial interest. Main visitors are Apidae (honeybees and bumblebees). For *S. inaequidens*, the related native species *Jacobaea vulgaris* was selected as a relevant competitor. Main visitors are Diptera, especially Syrphidae, and Hymenoptera.

In 2008, three experiments were carried out, each with a pair of one HIPS and one native species: i) *I. glandulifera – E. angustifolium*, ii) *I. glandulifera – A. napellus*, iii) *S. inaequidens – J. vulgaris*. We set up a design that combined three HIPS numbers (clumps of 0, 5 and 25 HIPS) with 2 distances (0 and 15 m) between HIPS and the native species which were arranged in clumps of 7 individuals (Figure 10). All plants were grown in pots. Each experiment was replicated on two different sites. Both sites were mown grassland exposed to the sun all day long (no shading from trees). For *S. inaequidens*, due to the limited number of flowering individuals, the experimental design was limited to one distance (0 m) between native and invasive species. In each clump of plants (native and HIPS), we recorded the number of visits, visitor identity and number of visited flowers or capitula (for Asteraceae) per trip during 10 min-periods at three periods per day: morning (10 to 11.30 a.m.), mid-day (12.30 to 14 p.m.), and afternoon (15 to 16.30 p.m.). Six repetitions were carried out (only sunny days). The visitation rate was calculated as the total number of visitors per 10 min divided by the number of open flowers or capitula. Seed set (number of seeds / number of ovules) was estimated on mature fruits. The shape and size of pollen grains of *E. angustifolium* and *A. napellus* differ from those of *I. glandulifera*, thus, we estimated pollen transfer by counting the native and the alien pollen grains deposited on native flower stigmas.

In order to validate the results from 2008, experiments with the *I. glandulifera*-native pairs were repeated in the summer of 2009. The experimental design of 2008 was adapted to add bigger clumps of HIPS (100 individuals; Figure 10). Alien pollen transfer was verified with fluorescent dyes (analogous of pollen, following methods in Kearns and Inouye 1993).

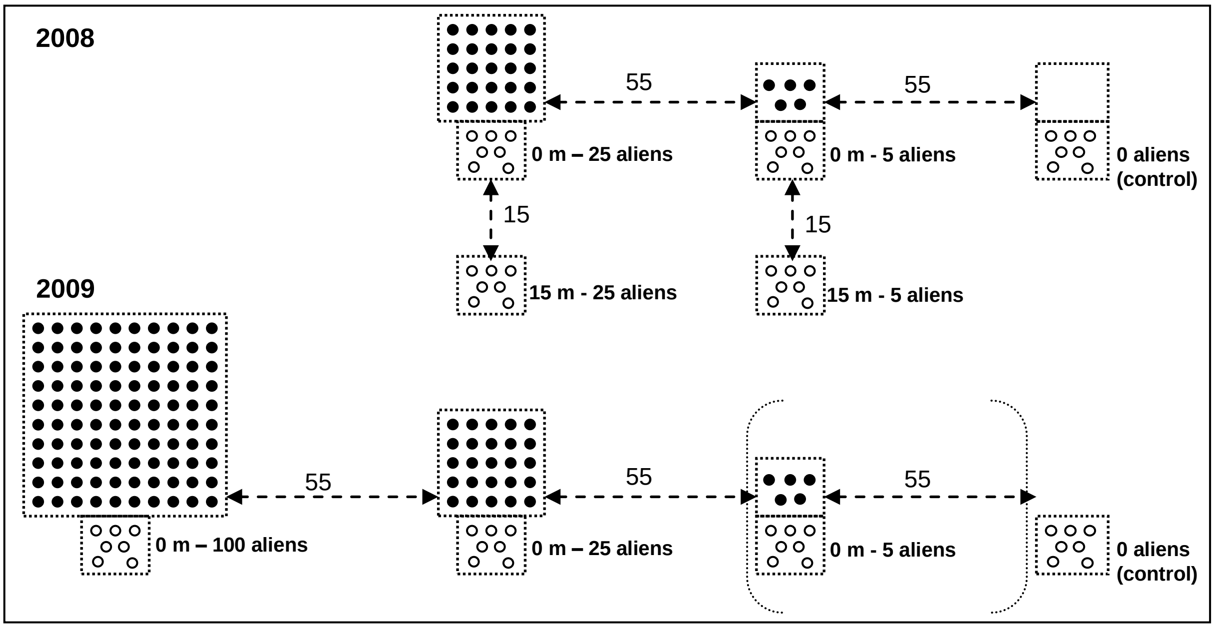


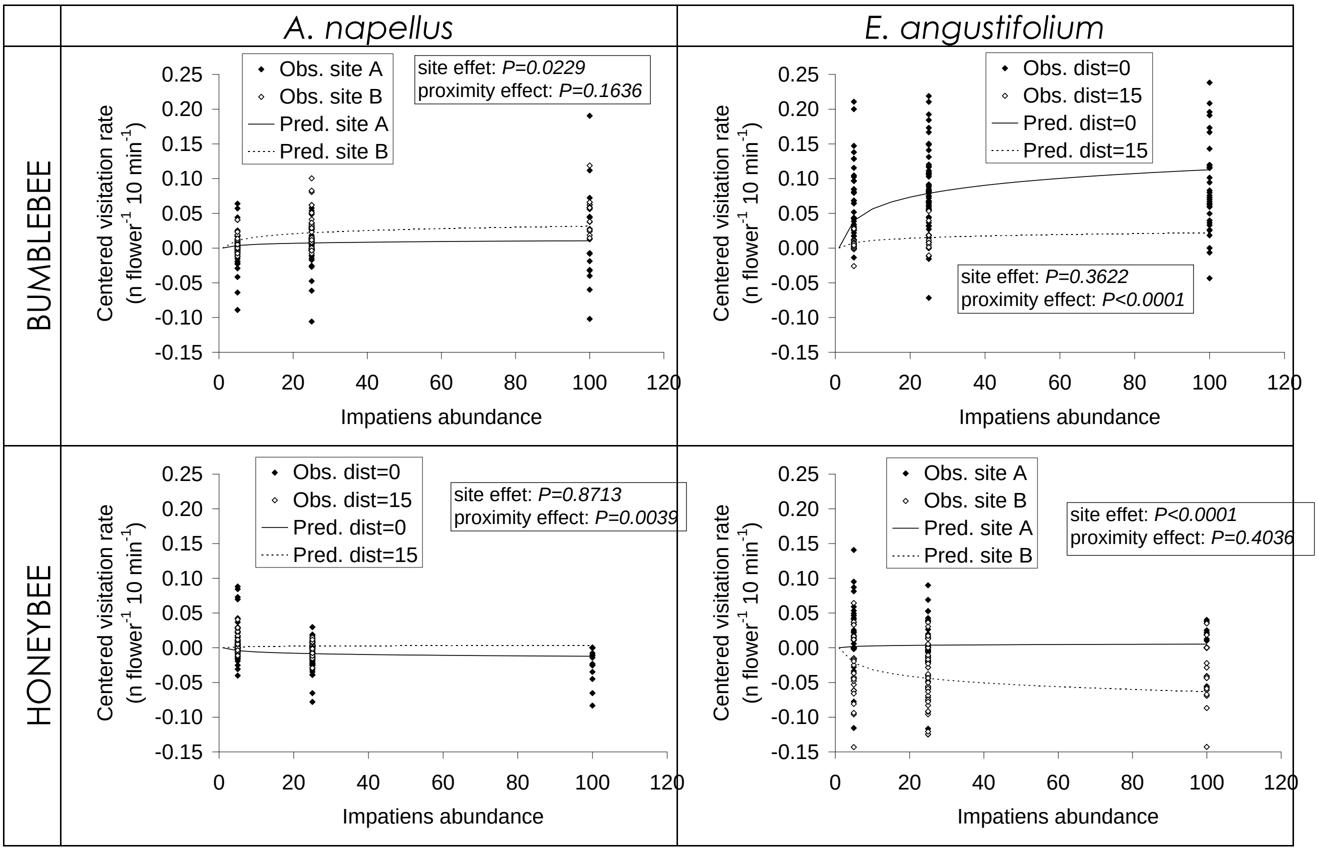
Figure 10: Experimental design for invasive-native pairs. Each dot represents one individual of alien (black dots) or native (white dots). Pair *S. inaequidens – J. vulgaris* only in 2008 and at the distance of 0 m. Pairs *I. glandulifera – E. angustifolium* and *I. glandulifera – A. napellus tested in* both years.

Results

S. inaequidens and *J. vulgaris* did not differ in the number of visitors per 10 min (about 8-9 visitors for both species; T-Test: T = -0.67, p = 0.50) but the visitation rate was significantly higher for *S. inaequidens* (0.7 *versus* 0.16; T=-5.4, p<0.001). The number of *S. inaequidens* plants had no impact on the visitation rate on *J. vulgaris* (two-way ANOVA, p = 0.341). Neither the effect of the site (p = 0.738) nor that of the interaction site*number of *S. inaequidens* (p = 0.354) was significant. For both species, insect foraging behaviour was similar between the two sites both in terms of number of visited capitula and time per capitulum (Kruskal-Wallis: χ^2 = 0.006, p = 0.98; χ^2 = 0.004, p = 0.95 respectively). Visitors switching between the two species were often observed leading to possible inter-specific pollen transfer. Nevertheless, the seed set of *J. vulgaris* was not significantly influenced by the presence of *S. inaequidens* (ANOVA, p = 0.10). No difference between sites was detected (p = 0.65).

The visitation rate to *I. glandulifera* (years and sites pooled, 0.362) was 5.6 times higher than to *E. angustifolium* and 9.8 times higher than to *A. napellus*. As the relative frequencies of insect categories varied between years for native plant species and in order to analyse data of visitation rate by insect category independently of the temporal effect, we created a model which standardized temporal variation in visitor guilds. A linear mixed model describing the variation of the centred visitation rate with the natural logarithm of the *I. glandulifera* abundance was adjusted for each native plant species and pollinator category. The centred visitation rate was calculated by subtracting the visitation rates of each treatment by those of the control (treatment without *I. glandulifera*); this centred variable has the advantage that it can be directly interpreted as the effect of the invasive.

Whatever the native species, the site and the degree of proximity, the total visitation rate (all insect categories taken together) was not affected by *I. glandulifera* abundance, except for the site A at close proximity (0 m) for *E. angustifolium* where a positive effect was observed (*i.e.* more visits; facilitation effect; Figure 11). Regarding *A. napellus*, a positive effect of *I. glandulifera* abundance was observed on bumblebee visitation rate and a negative one on honeybee and small-sized syrphid visitation rates in site B. No effects were observed in site A. For *E. angustifolium*, a positive effect of *I. glandulifera* abundance was noticed at both sites on bumblebee visitation rates and a negative one on honeybee visitation rate in site B. These effects were generally more pronounced when *I. glandulifera* was closer (Figure 11). Among native plants, facilitation effects (more visits) were higher for *E. angustifolium*.



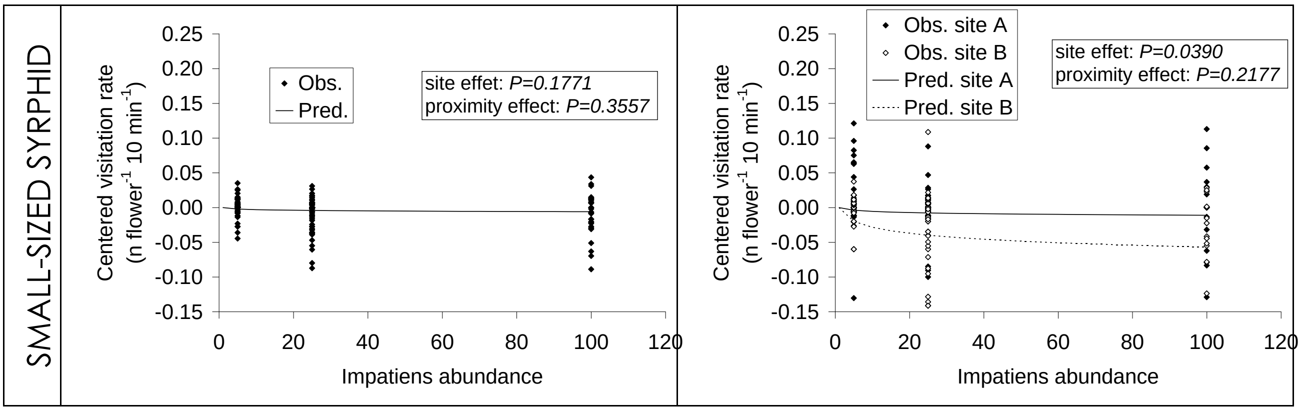


Figure 11: Centred visitation rate (observations and predictions) as a function of *I. glandulifera* abundance for both native plant species (*A. napellus* and *E. angustifolium*) and for the three main pollinator categories (bumblebee, honeybee and small-sized syrphid). On each graph, P values of the contrasts testing for site and proximity effects are presented. If the P value is significant for one of the two effects, the different levels of this effect are discriminated.

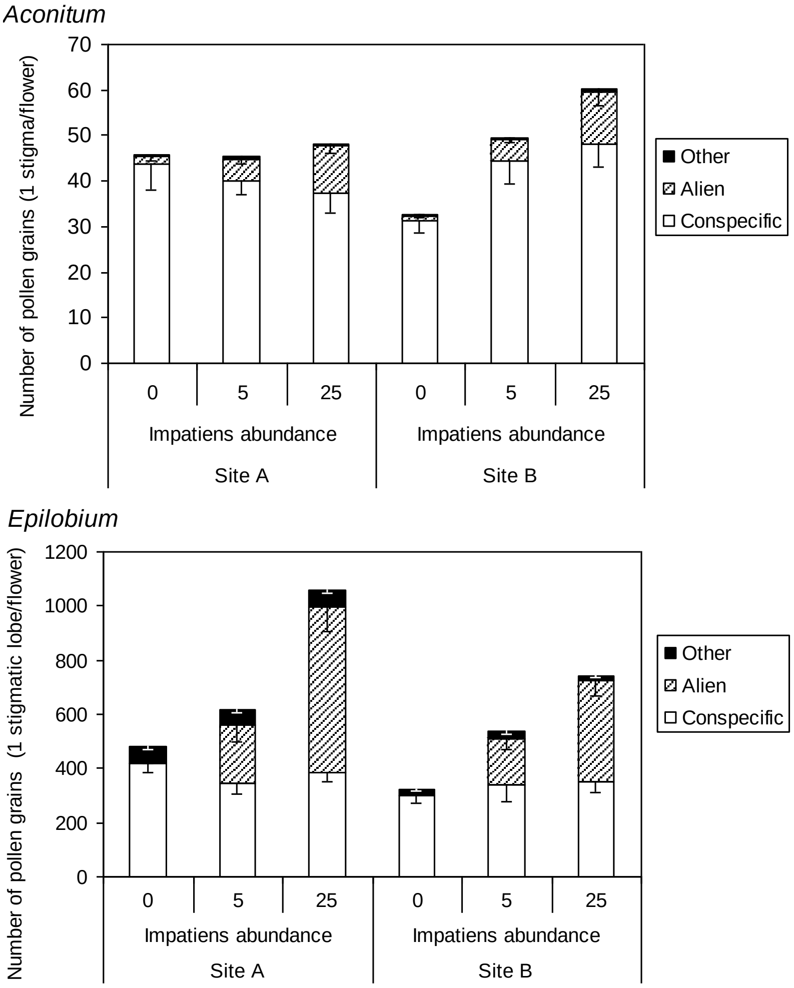


Figure 12: Origin (conspecific, alien or other) and quantity of pollen deposited on stigmas of native species (per flower, one stigmatic lobe for *E. angustifolium* and one stigma for *A. napellus*). Data were collected in 2008 in clumps adjacent to *I. glandulifera* (distances of 0 m). N = 12-15 flowers.

Observations of native stigmas in clumps adjacent to the alien indicated that pollen coming from *I. glandulifera* were dispersed and deposited on native stigmas in both sites (Figure 12) and years. Bumblebees, the principal shared visitors between the alien and the native species were liable to deposit alien pollen on native stigmas. In 2009, the record of bumblebee movements between *I. glandulifera* and the paired native species showed numerous shifts between species. For *E. angustifolium*, at least 54% of bumblebees came from the alien and 74% left for it (25% and 35% respectively for *A. napellus*). For both natives, the amount of alien pollen increased with *I. glandulifera* abundance (two-way ANOVA; *A. napellus*: $p<0.001$; *E. angustifolium*: $p<0.001$) but the quantity of conspecific pollen (*i.e.* from the same species) was not modified (Figure 12; two-way ANOVA; *A. napellus*: $p=0.4269$, *E. angustifolium*: $p=0.7829$).

Nevertheless, the alien presence did not alter the seed set of both native species in both years and sites (Table III). The distance and the alien abundance had no influence on the fruit set of natives (Table III). For both native species the pollination was successful because seed sets remained high (*E. angustifolium* > 53%, *A. napellus* > 71%; Table III). No difference between sites was detected (two-way ANOVA; *E. angustifolium*: $p = 0.4533$; *A. napellus*: $p = 0.6193$) whereas the seed set of *A. napellus* was slightly higher the second year ($p = 0.0276$; *E. angustifolium*: $p = 0.5493$).

Table III: Seed set of native species (mean percentage per clump ± SE, n = 5-7 individuals). For each experiment, significant differences between treatments were tested with a one-way ANOVA (significance at $P < 0.05$).

				A. napellus					
I. glandulifera proximity		0 m				15 m		F	p
I. glandulifera abundance		0	5	25	100	5	25		
2008	Site A	74 ± 6	70 ± 3	75 ± 7		73 ± 4	80 ± 4	0.41	0.7984
	Site B	71 ± 5	78 ± 4	75 ± 5		72 ± 5	78 ± 3	0.46	0.7609
2009	Site A	82 ± 3	85 ± 3	82 ± 3	78 ± 4			1.03	0.3980
	Site B	80 ± 4		81 ± 2	77 ± 4			0.19	0.8317
				E. angustifolium					
I. glandulifera proximity		0 m				15 m		F	p
I. glandulifera abundance		0	5	25	100	5	25		
2008	Site A	62 ± 3	57 ± 2	61 ± 4		64 ± 3	61 ± 1	0.46	0.7620
	Site B	55 ± 3	53 ± 3	60 ± 6		56 ± 1	56 ± 2	0.42	0.7937
2009	Site A	61 ± 3	62 ± 3	61 ± 4	56 ± 3			0.67	0.5183
	Site B	64 ± 3		58 ± 4	61 ± 3			0.79	0.4688

POLLINATION INTERACTIONS WITH AN AQUATIC HIPS: *L. GRANDILFLORA*

Material and methods

To determine impact of *L. grandiflora* on pollinator services of native plants, three sites (ponds) where chosen with a different cover (control – low – high) of the invasive species. For this part of the study, six potted *L. salicaria* plants were planted in a clump next to the *L. grandiflora* vegetation. Insect observations for both plants were performed on monospecific patches. Before each observation period the number of observed flowers in the monospecific patches was counted for each species. A total of 60 10 min-periods of insect observations were performed on dry and sunny days in three periods: morning (9.30 to 11.30 am), mid day (12.30 to 2.30 pm) and afternoon (3.30 to 5.30 pm). We recorded type of visitor and number of visitors. Each visitor was classified in one of the main categories: Apidae (*Apis mellifera, Bombus* sp.), other Hymenoptera, small-size Syrphidae, large-size Syrphidae, other Diptera, Lepidoptera and Coleoptera.

To determine heterospecific pollen transfer, fluorescent dyes were applied in the low cover and high cover sites. Dyes were applied with wooden toothpicks to dehiscing anthers of 40-59 flowers from *L. grandiflora* (= dye source plants). In the high cover site two dye source patches were used to test the effect of distance. Dye source 1 was immediately next to the native clump of *L. salicaria*, dye source 2 was at a distance of 55 m. After 1-3 sunny days a total of 890 stigmas were collected from native (*L. salicaria* and other flowering native species) recipient plants using map coordinates. At the end of the experiment five ripe fruits per native *L. salicaria* clump at each site were collected and seed set (number of seeds/plant) was calculated as a measurement for reproductive success.

Results

More insects were recorded on *L. salicaria* plants when the cover of *L. grandiflora* was low compared to the control plants (no cover site, Kruskal-Wallis: $\chi_2^2 = 12.41$, $P < 0.01$, Figure 13). The mean (± SE) number of visits per 10 min to *L. salicaria* was 7.25 ± 1.87 in the no cover site, 19.42 ± 2.53 in the low cover site and 12.92 ± 3.17 in the high cover site. Syrphids were the most abundant insect group observed (31%, Figure 14). The flowers of *L. grandiflora* were highly attractive to the pollinators in both the low (16.25 ± 2.61) and high cover site (38.75 ± 4.08, Figure 14). The pollinator assemblage of the alien species was dominated by *Apis mellifera* (64% visits, Figure 14). *L. salicaria* and *L. grandiflora* differed in the number of visitors per 10 min only in the high cover site (Mann-Whitney: U = 14.50, Z = -3.32, P < 0.001, Figure 14). The Proportional Similarity (PS) between the two plant species was 0.19 in the low cover site and 0.76 in the high cover site.

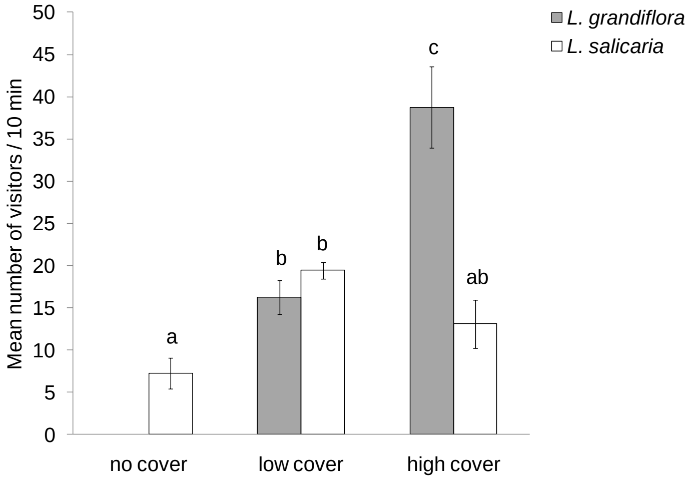


Figure 13: Number of pollinator visitors to invasive *L. grandiflora* and native *L. salicaria* (means ± SE) recorded in 10 min observations at three different densities of *L. grandiflora*. Different letters indicate significant differences.

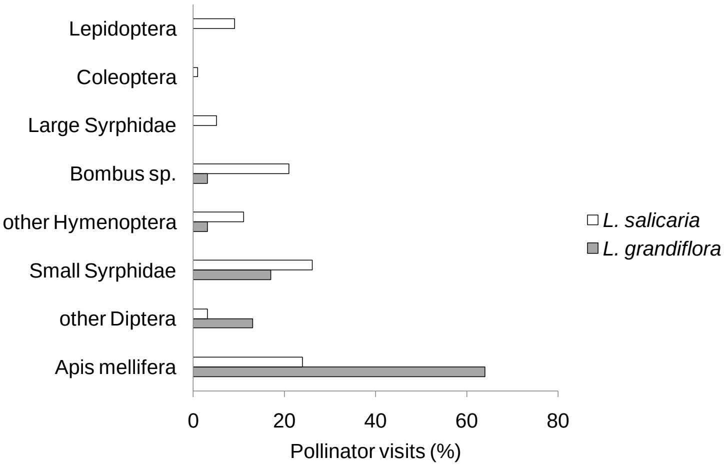


Figure 14: Pollinators observed on native *L. salicaria* and invasive *L. grandiflora* (the three densities were pooled). Number of insects recorded on *L. salicaria* and *L. grandiflora* was 470 and 652, respectively

Overall, only 6% of the native stigmas received dye. Most of these dye particles (73.5%) were transferred from *L. grandiflora* to the *L. salicaria* clump. There was only heterospecfic pollen transfer to the *L. salicaria* clump at short distance from the alien *L. grandiflora* dye source plants. In the low cover site 13.46% of the *L. salicaria* stigmas received dye compared to 47% in the high cover site. Seed numbers were lowest (96.8 ± 5.36) in the site with a high cover of *L. grandiflora* (high dye transfer) and slightly higher (112.4 ± 5.53) in the low cover site (facilitation effect) but no significant differences were found.

CONCLUSION

The potential impacts of HIPS on pollen transfer dynamics and subsequent seed sets of sympatric co-flowering natives, with which they share pollinators, have been increasingly studied these last years. Insect visitors of native plants are often generalists and they readily include aliens in their diets (Memmott and Waser 2002, Lopezaraisa-Mikel et al. 2007, Aizen et al. 2008, Vilà et al. 2009). Whatever the approach used in our study (plant-pollinators network or species-species comparisons) our results showed that terrestrial and aquatic species are highly attractive to a high number of native pollinators and are well integrated in native plant-pollinators networks. The main pollinators observed, honeybees, bumblebees, syrphids and flies, are typically generalists in their foraging choices. Extended resources offered by these mass-flowering generalist HIPS and the prevalence of generalist pollinators in natural communities may induce changes in pollinator services to native plants with consequences for their reproductive success.

The presence of *S. inaequidens* did not alter pollinator visits and seed set of the native relative species *J. vulgaris*. The alien did not turn the pollinators away from the native, even at high density and in close vicinity. Although visitors switched between the two species, no decrease in seed set of *J. vulgaris* was observed, suggesting that there is no pollen competition between the two plant species.

Similarly, no effects on insect visitation rates were observed in the native *E. angustifolium* and *A. napellus* in the presence of the alien *I. glandulifera*. Nevertheless, an increase of bumblebee visits to the natives was detected at high abundance and at short distance of the alien. These major visitors switched from *I. glandulifera* to natives and alien pollen was transferred to stigmas of the natives. This heterospecific transfer of pollen did not affect seed set in natives. This lack of negative impact on seed set was explained by the fact that native species were over-pollinated, they still received enough conspecific pollen grains to ensure high fertilization rate.

Little evidence of a strong effect of invasive *S. gigantea* populations on *D. carota* pollination was found in this study. However, approaching the problem at two spatial scales yielded contrasting results. If no difference was found between sites, a small-scale effect was detected on plant visitation by insects. The absence of difference between sites may be attributable to a larger diversity in flower composition and environmental variables, making differences less easy to detect. The lower visitation of natives at the invasive population edge indicates that competition for pollinators between the co-flowering species exists but is limited in space.

There is little evidence of negative impact of *L. grandiflora* on pollination of *L. salicaria* mediated through pollinators. On the contrary, we detected a weak facilitation effect of the invasive species at the low cover site. At the high cover site *L. grandiflora* was more efficiently visited by pollinators without turning them away from *L. salicaria* probably due to limited pollinator overlap. We could only detect heterospecific pollen transfer at short distance (1m) from *L. grandiflora*, a distance that frequently occurs in field populations.

Previous studies on plant-pollinator interactions presented ambiguous results with positive, neutral and negative effects (Bjerknes et al. 2007 and references therein). As a general pattern, for both terrestrial and aquatic HIPS, there was no strong evidence of impact. However, (weak) facilitation effects were detected for *L. grandiflora* and *I. glandulifera*. Invasive species acting as 'magnet species' have been found in other studies (Moragues and Traveset 2005, Larson et al. 2006, Nielsen et al. 2008).

The potential of one plant to affect pollination of a co-flowering species is greatest in immediate vicinity of that target plant (Grabas and Laverty 1999). In our experiments, there was limited heterospecific pollen transfer, even at short distance from the alien, and this did not affect the reproductive success of our tested native species.

Our results show that the modifications of pollination services to native species in native-invasive competition experiments were highly idiosyncratic and strongly depended on species pairs, sites, and seasons as has been shown by other studies (Roubik et al. 2001, Price et al. 2005).

INDIRECT IMPACT VIA SOIL MODIFICATION

Previous results (SSDII contract N° EV/11/27C) revealed significant effects of highly invasive plant species on topsoil nutrient pools. Therefore we examined the mechanistic basis for such impacts, especially the impacts of *F. japonica* on nitrogen (N) and *Solidago gigantea* on Phosphorus (P).

ORGANIC MATTER AND NITROGEN CYCLING IN *F. JAPONICA*.

Materials and methods

Litter decomposition rate was compared between *F. japonica* and native species, in two environments (invaded by *F. japonica* and uninvaded), in one site (SGH). Pools of C and N in the remaining material were monitored for one year. The initial lignin and cellulose content was also analysed. N Fluxes in the soil-plant system were indirectly assessed by monitoring seasonal variation in N pools in aboveground living biomass and litter. In two sites (ORN and TER), nitrogen mineralization rate was analysed by in situ incubation of undisturbed soil cores following the method of Olff et al. (1994). The mineralisation rate was calculated as the difference in nitrate and ammonium pools between the incubated core at time t and the control cylinder at time t-1.

In order to get deeper insight into the mechanisms involved in N cycling modification, we studied the influence of *Fallopia* spp. on nitrifying and denitrifying bacteria activity and abundance. The impact of *Fallopia* spp. on nitrifying (Ammonia and Nitrite oxidizing bacteria: respectively AOB and NOB) and denitrifying bacteria was assessed in ten sites (5 in Belgium and 5 in the region of Lyon, France). In vitro ammonia oxidizing enzyme activity (AOEA), nitrite oxidizing enzyme activity (NOEA) and denitrifying enzyme activity (DEA) were assessed. Total DNA extracts were used to assess the abundance of AOB, NOB and denitrifying bacteria with a quantitative PCR (qPCR) targeting a function gene of each functional group (respectively specific 16S rRNA, nxrA and nirK). For technical details, see Dassonville et al. (in press).

Results and discussion

The litter of *F. japonica* (especially stem litter) had much higher concentration of lignin and much lower concentration of N compared to the native litter, resulting in extremely high values of lignin:N ratio (Table IV). These results are confirmed by Aguilera et al. (2010). Native litter decomposed much faster than *F. japonica* litter (Figure 15). In addition, litter decomposition occurred at slightly higher rates in invaded stands but this effect was much smaller than the litter quality effect. The impact of *F. japonica* on decomposition appears to be overwhelmingly explained by the low decomposability of its litter while indirect modifications through altered microclimate and soil fauna seem negligible, in contrast to other invasive species (e.g. Lindsay and French 2004, Ashton et al. 2005).

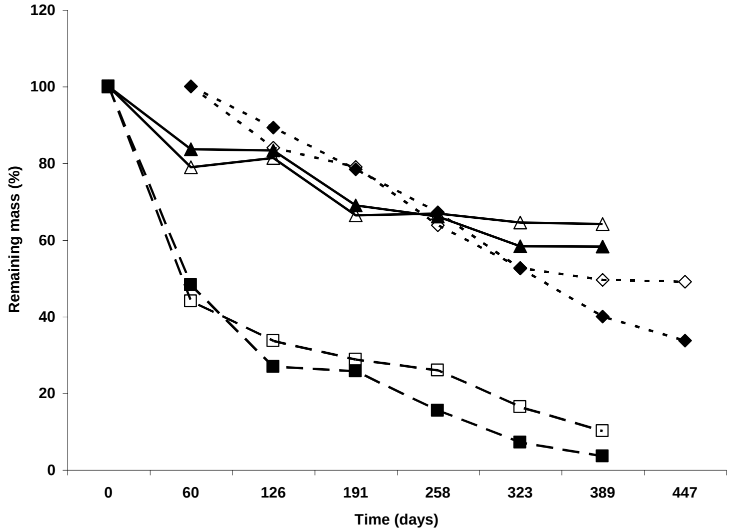
Table IV: Chemical composition of the three litter types. Mean ± SD (n = 2).

Litter type	C (%)	N (%)	C/N	Lignin (%)	Lignin:N	Cellulose (%)
***F. japonica* leaves**	45.7 ± 0.2	0.63 ± 0.01	72.5 ± 1.2	14.5 ± 0.04	23.1 ± 0.4	27.5 ± 0.18
***F. japonica* stems**	45.4 ± 0.3	0.30 ± 0.01	151.3 ± 5.1	18.8 ± 0.05	62.8 ± 2.1	53.8 ± 0.08
Native mixture	45.5 ± 0.2	1.38 ± 0.02	33.0 ± 0.5	12.1 ± 0.03	8.8 ± 0.1	23.2 ± 0.05

After one year, indigenous litter had lost more than 90 % of its initial N pool (not shown). In contrast N stocks in *F. japonica* litter had been increasing from 25.2 to 34.3 mg of N for leaves and from 12 to 14.3 mg of N for stems indicating net immobilization of N. These results are consistent with Maurel et al. (2010) who observed a thicker A layer, as well as a darker topsoil under *F. japonica*. Concerning N fluxes, a striking result is the huge amount of N allocated by *F. japonica* to shoots (79 g m^{-2} = 56 g m^{-2} to leaves and 23 g m^{-2} to stems and branches). Senescing leaves and stems retranslocated 79% of the aboveground N before abscission. This high resorption efficiency is in line with Aguilera et al. (2010). Total aboveground N stocks in *F. japonica* plots were always higher compared to uninvaded vegetation. It thus appears that a large amount of N is permanently blocked in dead and living matter in invaded plots, as also found by Aguilera et al. (2010).

In both sites, the nitrification rate was significantly reduced in invaded compared to uninvaded sites (Figure 16, significant in site TER). Since 98-99 % of the mineralised N was nitrified, the results suggest that *F. japonica* had a negative impact on ammonification rate, due either to direct inhibition of microorganisms implicated in the ammonification process or to lower quality of the organic matter. Whatever the mechanism, the net flux of mineral N released in the soil and available for plants is lower in invaded than in uninvaded soils. Our results contrast with Aguilera et al. (2010) who found no significant impact on N mineralization rate, because the low litter quality was possibly compensated by larger total amounts on N returned to the soil. The impact of *F. japonica* on N cycling may thus be site-dependent, which should be investigated in future research.

The results strongly suggest that efficient internal cycling of N, and negative impacts on N mineralisation due to production of recalcitrant litter, are key components of the impacts of *F. japonica* on soil N.



	df	MS (% of initial mass remaining)
Litter	2	42047.7 ***
Invasion	1	451.6 ***
Time	5	6627.4 ***
Litter*Invasion	2	96.5 **
Litter*Time	10	366.8 ***
Invasion*Time	5	255.5 ***
Litter*Invasion*Time	10	37.6 *

Figure 15: Decomposition kinetics of *F. japonica* leaves (triangles), stems (diamonds) and native litter (squares) during one year. All litter types were incubated in invaded (black) and uninvaded (white) environment. Decomposition is expressed as the percentage of initial mass lost. Values are means ± standard deviation. The table shows the results of a three-way ANOVA . Degree of freedom (df), Mean square (MS) and significance level: *: p<0.05, **: p<0.01, ***: p<0.001.

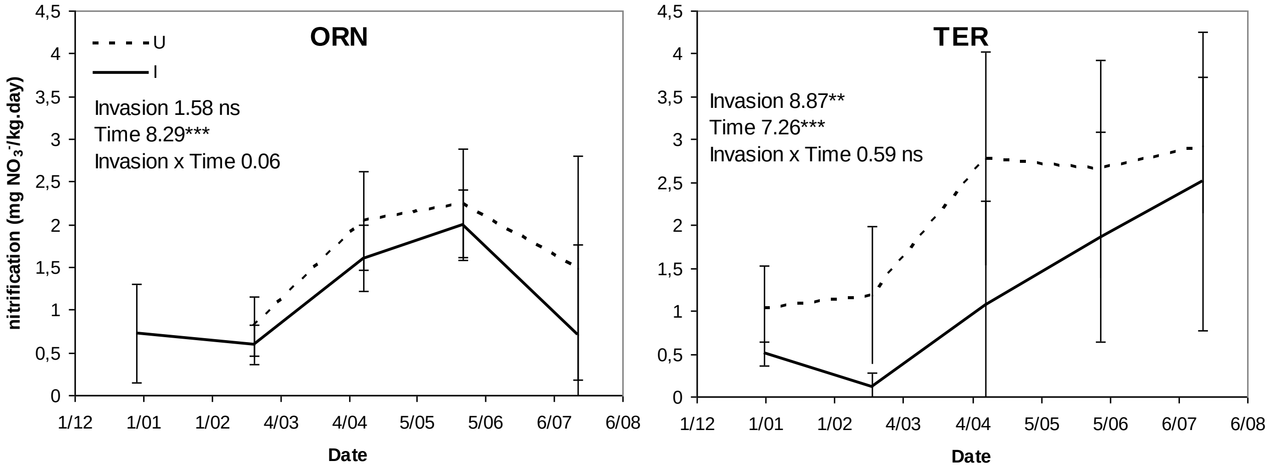


Figure 16: Mean daily nitrification rate in invaded (I) and uninvaded (U) soils of the site ORN. The dates are the mid time of incubation period; A: In the Orneau site (ORN), B: in the Tervuren site (TER). Error bars are standard deviation. ANOVA: F and significance level: *: p<0.05; **: p<0.01; ***: p<0.001; ns: not significant. The post-hoc Tukey test showed no significant difference between invaded and uninvaded situation at any date.

We found that *Fallopia* spp. decreased potential denitrification enzyme activity (DEA) by reducing soil moisture and reducing denitrifying bacteria density in the soil. The plant also reduced potential ammonia and nitrite oxydizing bacteria enzyme activities (respectively, AOEA and NOEA) in sites with high AOEA and NOEA in uninvaded situation. Modification of AOEA and NOEA were not correlated to modifications of the density of implicated bacteria. AOB and *Nitrobacter*-like NOB community genetic structures were significantly different in respectively two and three of the four tested sites while the genetic structure of denitrifying bacteria was not affected by invasion in none of the tested sites. Modification of nitrification and denitrification functioning in invaded soils could lead to reduced nitrogen loss from the ecosystem through nitrate leaching or volatilization of nitrous oxides and dinitrogen and could be considered as a niche construction mechanism of *Fallopia* spp. This indicates that invasion by *Fallopia* spp. may aggravate the impacts of N eutrophication in ecosystems.

MECHANISMS OF IMPACTS OF *S. GIGANTEA* ON SOIL PHOSPHORUS POOLS

Materials and methods

The study was conducted in one site (KRA). We compared the seasonal variation of labile inorganic and organic soil P pools, microbial P, phosphomonoesterase activity and pH between plots invaded by *S. gigantea* and uninvaded adjacent vegetation. P pools in standing biomass were also assessed. Aboveground vegetation samples (living leaves, living stems and litter) were collected at three contrasted phenological states and root samples were excavated on two dates. Biomass and P concentrations were used to calculate P pools and fluxes in the soil-plant system.

Results and discussion

Soil pH was significantly lower in invaded plots at all dates (Figure 17), which may explain the increased labile P pools (Resin-Pi, Bicarb-Pi and NaOH-Pi) (Figure 18). In summer, *S. gigantea* stands had much higher standing biomass compared to the control plots. The stocks of P in belowground organs showed a more than two-fold increase in autumn (from 2000 to 5200 mg/m^2), due to massive production of new fine roots. *S. gigantea* alters topsoil chemistry, thus enhancing P availability. However, it captures very efficiently available P in autumn, possibly due to a flush of fine root production. In Switzerland Scharfy et al. (2009) found that *S. gigantea* had no effect on soil pH and P availability, highlighting that conclusions on impacts are difficult to generalize.

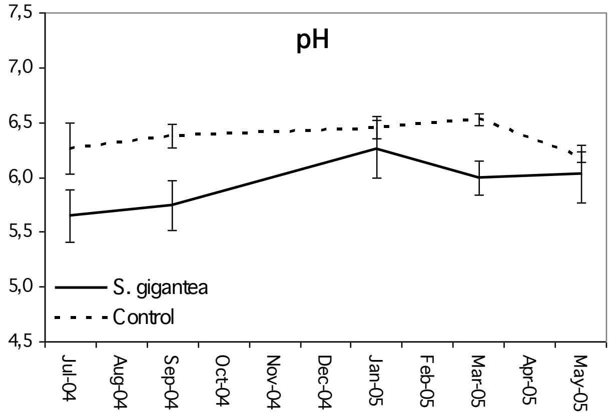


Figure 17: Seasonal variation of soil pH in plots invaded by *Solidago gigantea* and adjacent, uninvaded plots. Means (n = 6) and standard deviations.

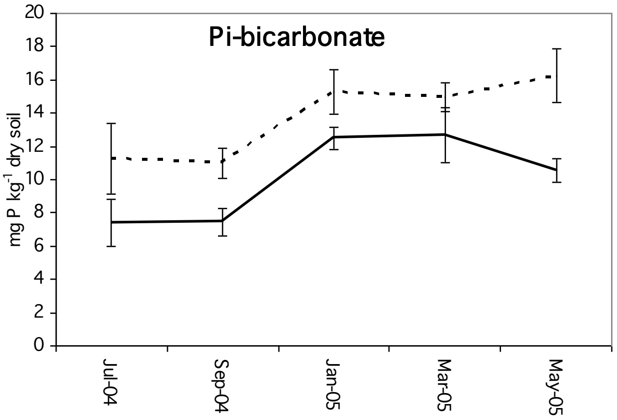


Figure 18: Seasonal variation of soil inorganic P extracted by bicarbonate (0-10 cm) in plots invaded by *Solidago gigantea* and adjacent, uninvaded plots. Means (n = 6) and standard deviations.

Conclusion

Highly invasive plants influence specific processes in the cycling of nutrients in the plant-soil system, resulting in alteration of pools of available nutrients in the topsoil. *F. japonica* produces highly recalcitrant litter that immobilizes N and internal recycling of N is enhanced. The result is that N availability to native species is strongly decreased. Manipulation of key limiting resources (N or P) appears to play a prominent role in the competitive superiority and the invasive success of both *F. japonica* and *S. gigantea*.

IMPACT OF SOIL MODIFICATION ON NATIVE AND ALIEN SPECIES COMPETITION

The objective was to test the hypothesis of a positive feedback of *F. japonica* on its own competitive success by the modification of soil properties.

Materials and methods

A competition experiment between *F. japonica* and a native species (*C. arvense*) was conducted in 5 L-pots (diameter: 15 cm, height: 30 cm) in three soil treatments: topsoil from plots invaded by *F. japonica*, soil from uninvaded plots and soil from invaded plots amended with charcoal (absorption and inhibition of allelopathic substances). Two pure cultures and a 50:50 mixture were used with 10 replicates (pots) per treatment (total = 90 pots). Rhizomes of the two species were collected in March 2008 and cut into pieces of 2 cm. All the rhizomes were planted simultaneously at the same density (4 plants per pot). Non-destructive growth measurements (number of leaves, axes length (main stem + ramifications), length and width of the largest leaf) were performed every three months.

Results and discussion

The ANOVA showed a significant soil x culture type interaction for the shoot height for *C. arvense* in June ($p<0.001$) and September 2008 ($p<0.01$) (Figure 19). *C. arvense* grew better in pure culture on invaded and uninvaded soil while the opposite pattern occurred in invaded soil amended with charcoal. The pattern observed for shoot height was also found for the number of leaves and the length and width of the largest leaf (not shown). There was a slight tendency for *F. japonica* to be negatively affected by the native species only in the presence of charcoal but this was not significant.

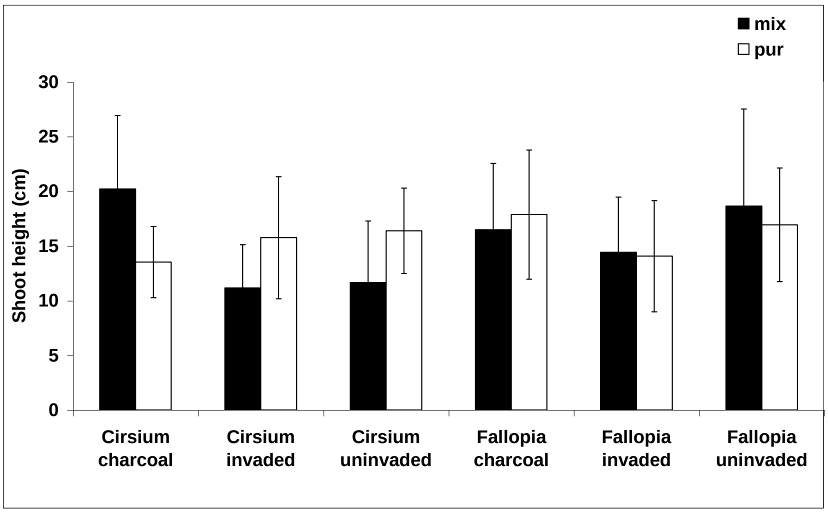


Figure 19: Mean shoot height of *Cirsium arvense* and *F. japonica japonica* in function of three soil treatments (preconditioned by *F. japonica,* not preconditioned, preconditioned amended with charcoal) in pure and mixed culture. Whiskers are standard error.

In conclusion, *C. arvense* won the competition on amended soil while it lost competition on invaded and uninvaded soil. There is some evidence that negative effects of *F. japonica* on native species are mediated by allelopathic compounds. However, there is no evidence for long lasting negative effects of allelopathic substances.

Parepa et al. (2010) found that *F. japonica* had an allelopathic effect on native forbs with a suppressive effect of *F. japonica* reduced by 35% in presence of activated charcoal.

C. IMPACTS AT OTHER TROPHIC LEVELS

Our objective is to test whether highly invasive plant species modify the soil faunal community structure in terrestrial ecosystems and the invertebrate, phytoplankton and zooplankton abundance, taxa richness and taxa diversity in aquatic ecosystems.

TERRESTRIAL

Materials and methods

Four species were used: *F. japonica*, *S. gigantea*, *I. glandulifera* and *S. inaequidens* with three sites per species. Hypogeic fauna was sampled with the Berlèse-Tulgren method. For the surface active macrofauna (epigeic), pitfall traps were used. All invertebrates collected were counted and identified at the family level and at the species level for the macrofauna (Diplopods, Isopods, Hymenoptera). The data are presented as counts of individuals for each taxonomic group, and total faunal density. A one-way ANOVA with invasion as fixed factor was conducted to compare the total number of individuals in invaded and uninvaded plots. A two-way ANOVA was made with invasion and site as fixed factors. Finally, a PCA including the tree sites was performed in order to see how the total faunistic assemblage differed between sites and between invaded and uninvaded situations.

Results and discussion

Only data for the hypogeic fauna are shown. Whenever large impacts were found in the epigeic fauna they are mentioned.

F. japonica

The total number of individuals was lower in invaded than in uninvaded plots but only significantly in the first site (SGH) (Table V). This decrease might be due in part to the low palatability of *F. japonica* litter. A recent study also found a 40% decrease of invertebrate abundance in *F. japonica*-invaded plots (Gerber et al. 2008). One explanation was the low palatability of *F. japonica* leaves. In the first site (SGH), the strong impact of *F. japonica* can be explained by the species-rich uninvaded vegetation (26 species vs 2-3 native species in the other sites). This indicates that the magnitude of impacts of *F. japonica* on soil fauna are larger when species rich sites are invaded. In all three sites, Oribatida and Hymenoptera were les abundant under *F. japonica* stands (Oribatida: SGH: 320 (total number of individuals) vs 824; TER: 77 vs 146; ORN: 113 vs 215; Hymenoptera: SGH: 0 vs 88; TER: 0 vs 32; ORN: 1 vs 30). As Oribatida are mainly detrivorous, the recalcitrant litter of *F. japonica* might explain their decreased density in invaded plots.

Table V: Impacts of *F. japonica on* soil fauna. Total number of individuals for the hypogeic and epigeic fauna in invaded and uninvaded plots in three sites. The table shows the results of t tests.

	Hypogeic			**Epigeic**		
	Site SGH	**Site TER**	**Site ORN**	**Site SGH**	**Site TER**	**Site ORN**
Uninvaded	1407	371	403	1620	1030	809
Invaded	532	242	278	581	880	677
T test	t=-2,95; **	t=-2,74; ns	t=1,48; ns	t=3,49; **	t=1,33; ns	t=1,25; ns

In the SGH site two groups (Isopoda and Diplopoda) were significantly more abundant under *F. japonica* and two others groups (Homoptera and Hymenoptera) were absent in invaded plots. The first two groups have affinity for shaded and moist environments. The absence of differences between those groups in the other sites may be due to the shaded and moist conditions existing in the native vegetation. Concerning the soil fauna assemblage, a clear distinction appeared in the SGH site (Figure 20). This might be explained by Isopods or Diplopods abundant under *F. japonica,* which have affinity for moist environments opposed to groups absent in uninvaded plots (Hymenoptera). Microclimate modification by *F. japonica* might explain impacts on soil fauna assemblage. In the two other sites, assemblages are less different between invaded and uninvaded plots, most likely because the latter have a woodland vegetation.

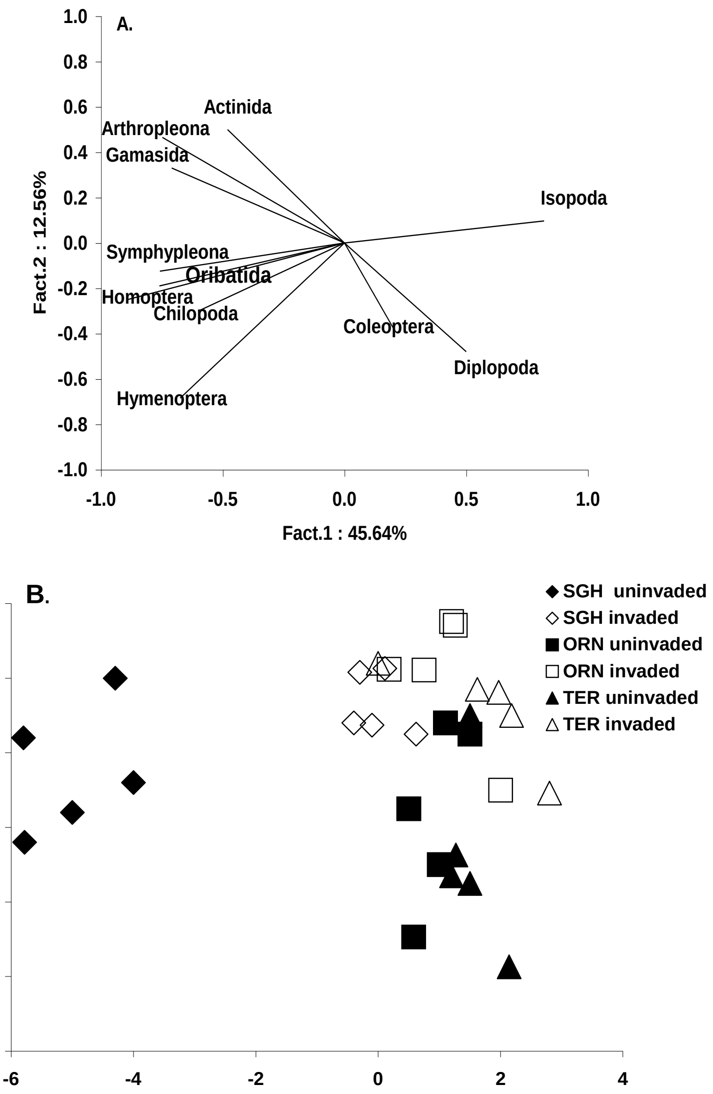


Figure 20 : Hypogeic faunal assemblage under *F. Japonica japonica*. Principal Component Analysis (PCA). A: Projection of variables (taxonomic groups) on PC1 and PC2, all sites pooled. B: Projection of the sampling points from the invaded (white) and the uninvaded (black) plots.

In conclusion, the impacts of *F. japonica* on soil fauna are more important in open than in closed vegetation. This result corroborates a recent study in Switzerland (Gerber et al 2008).

I. glandulifera

Population density significantly increased at all sites (table VI). This increase is mostly explained by Arthropleona springtails ($p < 0.001$). These springtails are mainly fungivorous (Maraun et al. 2003) and their increased density may be mediated by enhanced development of mycelia in *I. glandulifera* litter, possibly favoured by decreased pH values in invaded stands (Dassonvile et al.

2008). The two way ANOVA revealed an invasion effect for one group, Poduromorpha, which was more abundant in invaded plots ($p = 0.01$). This is another fungivorous group which feeds on fungal spores, again pointing to fungal development as a possible mediator of impacts on soil fauna. From Symphila for the epigeic fauna ($p < 0.001$) and Coleoptera ($p = 0.03$) and Oribatida ($p < 0.001$) for endogeic fauna were more individuals in invaded plots. Those groups are detritivorous (Coineau et al. 1997) suggesting that the litter of *I. glandulifera* might be more palatable. In site MUI, aphids were very abundant in uninvaded vegetation but totally absent in invaded plots, possibly due to an insecticidal and repellent activity of *I. glandulifera* (Pavela et al. 2009) Finally, three groups were differently affected by *I. glandulifera* depending on the site: Diploura, Symphila and Actinida (significant Invasion x Site interaction).

Table VI: Impacts of *Impatiens glandulifera* on soil fauna. Total number of individuals for the hypogeic and epigeic fauna in invaded and uninvaded plots in three sites. The table shows the results of t tests.

	Hypogeic			**Epigeic**		
	Site MUI	**Site TEM**	**Site KES**	**Site MUI**	**Site TEM**	**Site KES**
Uninvaded	395	469	342	248	258	220
Invaded	502	711	476	402	496	372
T test	t=1,32 ; ns	t=3,85; ns	t=0,67; ns	t=-9,57; ***	t=-4,34; **	t=-3,05; *

The PCA revealed a distinction between invaded and uninvaded plots (Figure 21). This is mainly due to the increased abundance of Arthropleona and Poduromorpha in invaded plots.

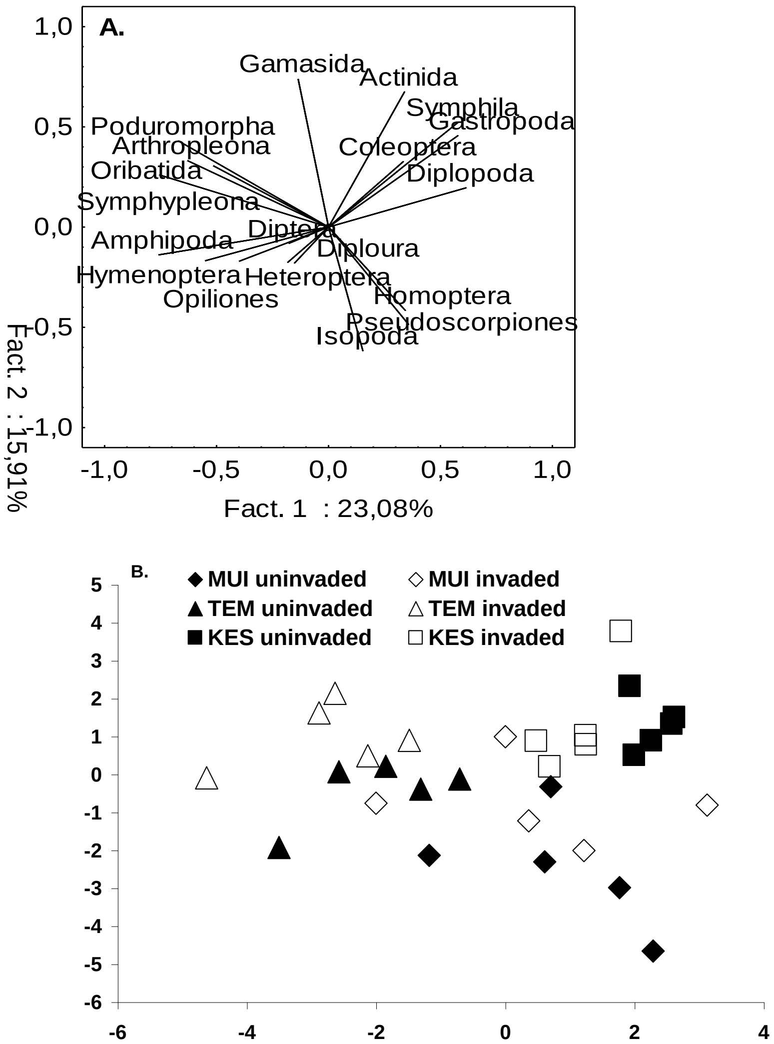


Figure 21 : Hypogeic faunal assemblage under *Impatiens glandulifera*. Principal Component Analysis (PCA). A: Projection of variables (taxonomic groups) on PC1 and PC2, all sites pooled. B: Projection of the sampling points from the invaded (white) and the uninvaded (black) plots.

S. gigantea

No significant change in total density was observed at any site (table VII). Isopoda had a significantly higher density in invaded plots (p = 0.003). This group has affinity for shaded and humid environments (Horowitz 1970). An invasion effect was also found for the Symphila (p = 0.003) which was lacking in invaded plots. This group has an affinity for dry environments (Chinery 2005), again pointing to microclimate as the mediator of the impacts of *S. gigantea*.

Table VII: Impacts of *Solidago gigantea* on soil fauna. Total number of individuals for the hypogeic and the epigeic fauna in invaded and uninvaded plots in three sites. Results of t-tests.

	Hypogeic			**Epigeic**		
	MLD	**FOR**	**KRA**	**MLD**	**FOR**	**KRA**
Uninvaded	197	181	140	167	203	258
Invaded	148	141	96	171	193	181
T test	t=0,97; ns	t=0,67; ns	t=1,29; ns	t= -0,13; ns	t= -0,24; ns	t= 1,77; ns

Large shifts in faunal assemblages are observed at all sites, mostly explained by Isopoda and Symphila. Figure 22 also provides some evidence for convergence of faunal assemblage in invaded plots.

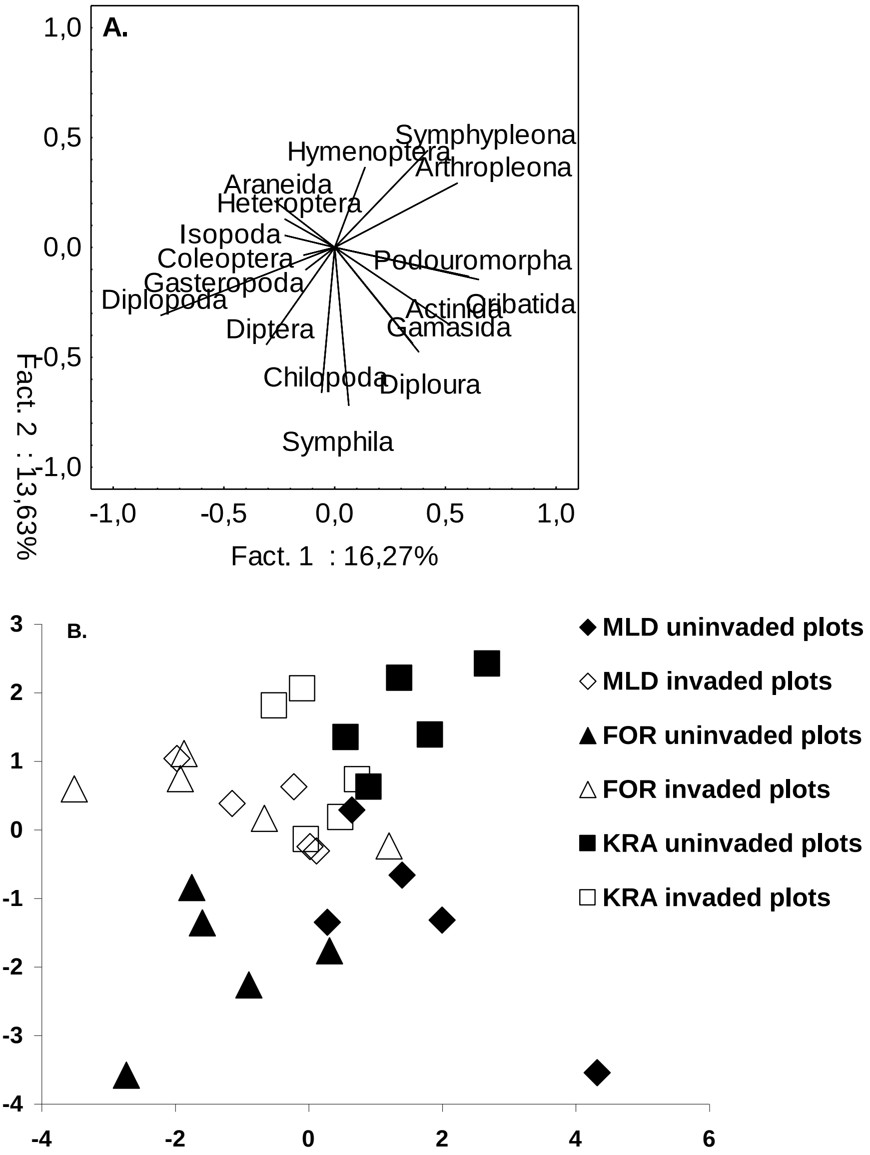


Figure 22: Hypogeic faunal assemblage under *Solidago gigantea*. Principal Component Analysis (PCA). A: Projection of variables (taxonomic groups) on PC1 and PC2, all sites pooled. B: Projection of the sampling points from the invaded (white) and the uninvaded (black) plots.

S. inaequidens

Total faunal density significantly decreased in two of three sites (Table VIII). This is mainly explained by Arthropleona (P < 0.001) and Gamasida (p = 0.003). Arthropleona feed mostly on fungi (Briones 1999). Their lower abundance might be due to antifungal properties of *S. inaequidens* (Loizzo et al. 2004).

Table VIII: Impacts of *Senecio inaequidens* on soil fauna. Total number of individuals for the hypogeic and the epigeic fauna in invaded and uninvaded plots in three sites. The table shows the results of t-test.

	Hypogeic			**Epigeic**		
	Site ANV	**Site HAR**	**Site KOE**	**Site ANV**	**Site HAR**	**Site KOE**
Uninvaded	913	478	384	655	437	381
Invaded	679	284	381	504	283	261
Test t	t=2,84; *	t=3,39; *	t=0,11; ns	t=2,15; ns	t=5,04; **	t=2,74; *

An invasion effect was found for two groups which more abundant in uninvaded soil, i.e. Isopoda (p = 0.02), and Diplopoda (Hypogeic fauna: p = 0.01; epigeic fauna: p < 0.001). Those groups prefer shaded and humid environments. The PCA shows that the magnitude of faunal assemblage c varies with site (Figure 23).

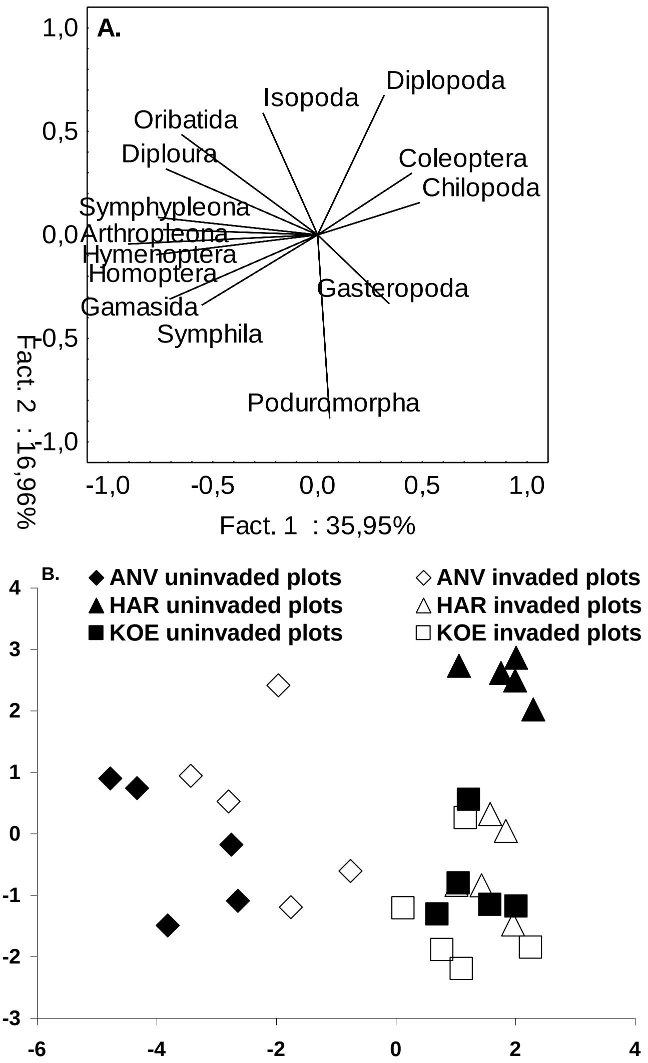


Figure 23: Hypogeic faunal assemblage under *Senecio inaequidens*. Principal Component Analysis (PCA). A: Projection of variables (taxonomic groups) on PC1 and PC2, all sites pooled. B: Projection of the sampling points from the invaded (white) and the uninvaded (black) plots.

Conclusion

Replacement of species-rich native vegetation by monospecific stands of invasive plants alters soil faunal assemblage. Modifications of soil fauna are species-specific and vary with site. The four studied species had significant impacts on soil fauna, but the patterns of impact are complex. For *S. gigantea* and *F. japonica,* the impacts are mostly mediated by alterations in microclimate. For *I. glandulifera* and *S. inaequidens*, litter chemical composition is apparently more important. Future studies should assess impacts at lower taxonomic levels (species) and should examine recovery of faunal assemblage after eradication.

AQUATIC

Material and methods

The study sites used for the impact study on other trophic levels represent a subset of the water bodies used for the impact study on native vegetation. A subset was chosen because of technical problems e.g. mechanical removal of the invasive species present or some ponds were too deep to take representative sediment samples with a hand-held coring tube.

Macroinvertebrates were sampled in invaded ponds and in adjacent uninvaded ponds using a 1 m long, hand-held corer. Six cores of 5 cm length (volume of 237.5 cm^3 sediment per core) were taken at each sampling site and pooled in the field. The corer was inserted into random aquatic plant patches beneath young submerged leaves or stolons and decomposed litter at approximately 1 m pond depth. Both macrophyte-dwelling invertebrates and invertebrates living in or on the sediment were sampled, but were not considered separately. Each subsample was placed into separate containers and preserved in 10% formaldehyde. In the laboratory each subsample was rinsed and passed through different mesh sizes (2 mm, 1 mm, 500 µm, 350 µm). Using a binocular, macroinvertebrates were separated from debris, enumerated, identified to family level and placed into a vial containing 70% ethanol.

Mixed water samples based on 6-10 random subsamples of 1L were taken from each invaded/uninvaded pond for phytoplankton and zooplankton analysis with a plastic tube sampler (4.5 cm diameter, 70 cm length) that closes in the lower part. For phytoplankton, the samples (500 mL) were fixed in the field with alkaline lugol, sodium thiosulfate and buffered formalin and stored at room temperature in the dark before identification to genus level and enumeration using inverted microscopy. For zooplankton, the samples were filtered through a 64 µm-mesh net and preserved in 5% formaldehyde (final concentration) at 4 °C before being identified and enumerated using inverted microscopy. Different levels of identification were used: cladocerans were identified to genus level, copepoids were divided into cyclopoids, calanoids and nauplii, rotifers were counted.

Invertebrates/phytoplankton/zooplankton abundance and taxa richness were analysed per invasive species to determine impact of invasion. Uninvaded ponds were grouped together. Standard Bray-Curtis similarity was calculated between the four types of vegetation. At pond level, a

Pearson/Spearman correlation analysis was performed between invasive species cover and abundance of invertebrates/phytoplankton/zooplankton. Bray-Curtis analysis was performed with PRIMER v6, all other analyses were performed using STATISTICA v8.

Results

Impact on invertebrates

Uninvaded ponds harboured in total 17 different families compared to 10, 9 and 14 families for *H. ranunculoides*, *L. grandiflora* and *M. aquaticum* invaded ponds, respectively. There was a considerable overlap in taxonomic composition of invertebrates in invaded and uninvaded ponds (range 52.16% - 79.69% similarity) with Diptera and Oligochaeta as the largest groups. Naididae, Chironomidae and Asellidae were the common families in the four vegetation types. Invaded ponds (either *H. ranunculoides, L. grandiflora* or *M. aquaticum*) were dominated by Naididae (56% or more). In uninvaded ponds Naididae (44%) and Planorbidae (29%) were the most abundant groups. Importantly, no mayflies were found in invaded ponds, while in uninvaded ponds several individuals of *Caenis* sp. And *Cloeon* spp. were observed. Correlation analysis showed that when invasive species cover increased, invertertebrate abundance significantly decreased among all ponds ($n = 22$, $r = -0.46$, $P < 0.05$) as well as among invaded ponds only ($n = 15$, $r = -0.58$, $P < 0.05$).

Impact on phytoplankton and zooplankton

Uninvaded ponds harboured in total 69 different genera of phytoplankton compared to 49, 55 and 56 genera for *H. ranunculoides*, *L. grandiflora* and *M. aquaticum* invaded ponds, respectively. Uninvaded ponds and *L. grandiflora* invaded ponds were dominated by Chlorophyta (34% and 57% respectively). In *H. ranunculoides* invaded ponds, Chlorophyta (26%) and Euglenophyta (27%) were the most abundant groups, while Cryptophyta (39%) was the dominant group in *M. aquaticum* invaded ponds. Similarity ranged from 47.5% to 59.5% between invaded and uninvaded ponds. No significant correlation could be detected between total number of phytoplankton cells and invasive species cover, however phytoplankton density tended to be higher in heavily invaded ponds (>50% cover). In uninvaded ponds a total of 9 different microcrustacean taxa were identified compared to 7, 9 and 7 taxa for *H. ranunculoides*, *L. grandiflora* and *M. aquaticum* invaded ponds respectively. The absolute microcrustacean abundance tended to be lower when alien species cover increased ($r = -0.68$, $P < 0.05$). Within the crustacean community, rotifers numerically dominated (69%-82%), except in the *M. aquaticum* invaded ponds, where the nauplii (copepods) were the largest group (42%). Similarity between uninvaded and invaded ponds was high (47.6% - 84.4%).

Discussion

For the different trophic levels there was no clear support for a considerable difference in overall species diversity at study sites covered by an invasive species when compared to non-covered sites. Our results on macroinvertebrates showed that *H. ranunculoides*, *L. grandiflora* and *M. aquaticum* cover negatively affected macroinvertebrate abundance in all ponds and even in invaded ponds only. At the time of our macroinvertebrate sampling, decomposed litter of these plants and sediments formed a dense dark mat on the bottom of the heavily invaded ponds (I. Stiers, personal observation; Dandelot et al. 2004). We hypothesize that this is a condition unsuitable for macroinvertebrate colonization resulting in lower abundance of macroinvertebrates when percent invasive species cover increased. Due to their physical presence these invasive species form dense canopies which occupy a large amount of space, greatly block sunlight and oxygen exchange. At some of our heavily invaded ponds, dissolved oxygen fell below 50% saturation. Under the litter of the invasive species, dipterans of the genus *Chironomus* and naidid oligochaetes were common and are known to be able to tolerate oxygen stress (Mandaville 2002). Taxonomic compositions of aquatic macroinvertebrate assemblages in invaded ponds differed from uninvaded ponds. Sensitive benthos, such as mayflies, were completely absent in invaded ponds. The decreased levels of oxygen under the canopy may also be adverse for zooplankton abundance. The higher phytoplankton density in heavily invaded ponds might be due to the entrapment capacity of the invasive species as has been proposed for invasive *Eichhornia crassipes* which has a similar growth form (Brendonck et al. 2003).

CONCLUSION

For both terrestrial and aquatic communities it was a common trend to observe a decrease in the total number of individuals when communities were invaded (except for *I. glandulifera*). This could be explained by differences in microclimate between invaded and uninvaded environments (terrestrial), the quality of litter, which was unsuitable for colonisation (terrestrial and aquatic) and the decrease of plants diversity in invaded plots compared to the native vegetation (terrestrial and aquatic).

D. FACTORS THAT MODIFY IMPACT

EFFECTS OF EUTROPHICATION ON COMPETITION BETWEEN INVASIVE AND NATIVE SPECIES

We investigated the effect of soil eutrophication on the competitive balance between native and three invasive alien plant species (*F. japonica*, *S. gigantea* and *S. inaequidens*) and of water eutrophication on the competition between the invasive *L. major* and the native *C. demersum*.

TERRESTRIAL

Materials and Methods

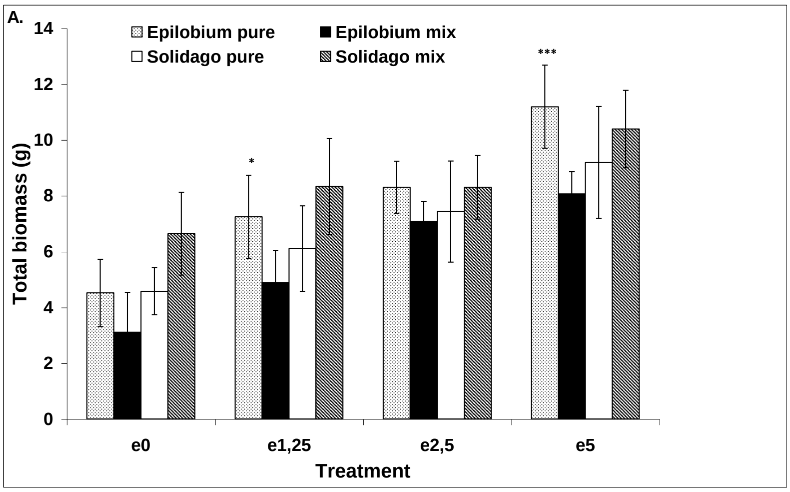
To test if competitive superiority of invasive plant species on the native flora is modulated by soil nutrient fertility, a competition experiment in pots was conducted for three species pairs (invasive vs. native): *F. japonica* vs. *C. arvense*, *S. gigantea* vs. *Epilobium hirsutum* and *S. inaequidens* vs. *Plantago lanceolata*. The three selected native species are widespread in native vegetation at invaded sites. Four soil fertility treatments have been created by additions of NPK fertiliser (16-18-25) to a sandy soil (0 g, 1.25 g, 2.5 g and 5 g per pot). Per species pair, two pure cultures and a 50:50 mixture were grown in containers. After 2 years (August 2010), the plants were harvested and weighed. For each pair of species separately, a two-way ANOVA was performed on total biomass with fertilisation and competition (pure or mixed) as fixed factors.

Results

In the first species pair, the invasive *S gigantea* was competitively superior to the native *E. hirsutum*, and this was most obvious at the highest fertility level (Figure 24A). There was no significant competition x fertility effect, indicating that eutrophication did not significantly alter the competitive relationships between the two species.

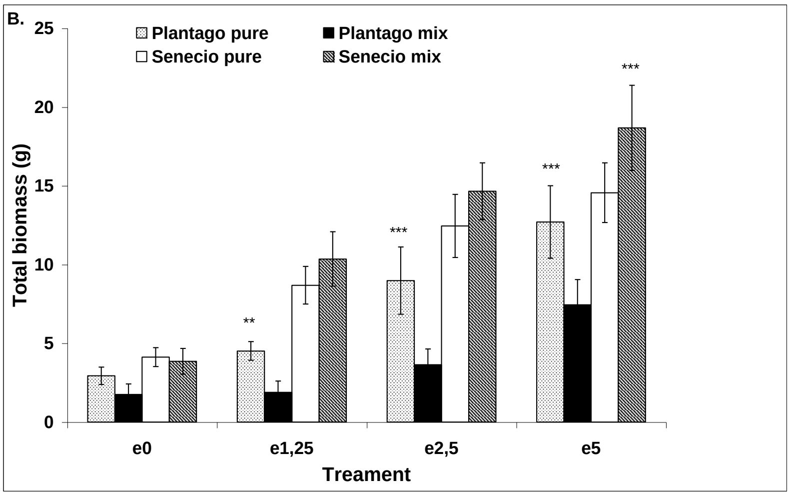
Also in the second species pair, the alien invasive species (*S. inaequidens*) was competitively superior to the native (*P. lanceolata)* (Figure 24B). A significant fertility x competition interactions for both species indicates that competitive asymmetry was more expressed at moderate and high fertility.

In the third species pair, *C. arvense* did not respond significantly to fertility, opposite to *F. japonica* (Figure 24C). *C. arvense* was not affected by competition with *F. japonica*, at any fertility treatment. Surprisingly, *F. japonica* was negatively affected by competition with *C. arvense*, and this effect strongly enhanced at high fertility levels.



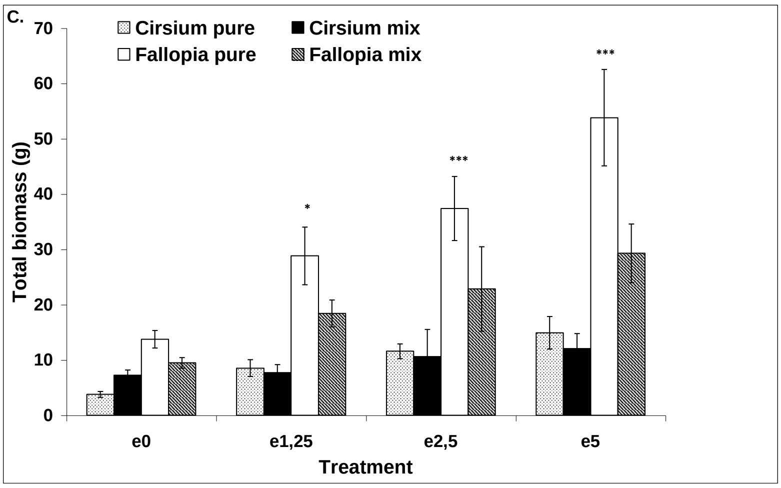
Epilobium	
Competition	46,26 ***
Fertilization	76,66 ***
Competition * Fertilization	2,51 ns

Solidago	
Competition	18,78 ***
Fertilization	23,09 ***
Competition * Fertilization	0,79 ns



Plantago	
Competition	112,14 ***
Fertilization	104,81 ***
Competition * Fertilization	9,08 ***

Senecio	
Competition	20,28 ***
Fertilization	152,97 ***
Competition * Fertilization	5,79 **



Cirsium	
Competition	0,21 ns
Fertilization	31,87 ***
Competition * Fertilization	4,66 **

Fallopia	
Competition	91,14 ***
Fertilization	81,02 ***
Competition * Fertilization	9,34 ***

Figure 24: Competition between one invasive and one native species at four fertility levels. Total biomass (g) (aboveground + belowground) (mean + SD) A: *Solidago gigantea* and *Epilobium hirsutum*; B: *Senecio inaequidens* and *Plantago lanceolata* C: *F. japonica japonica* and *Cirsium arvense* (four different applications : 0 – 1,25 – 2,5 – 5 g of NPK fertilizer). Plants were cultivated in pure and mixed culture with four plants per pots and 10 replicates per treatment. Whiskers are standard deviation. Table shows F values from a two way ANOVA with competition and fertilization as fixed factors: *: p<0.05, **: p<0.01, ***: p<0.001. Significant levels on the graph results from a Tukey post hoc test.

Discussion

Our results do not support the hypothesis that eutrophication consistently shifts the competitive balance in favour of invasive species. Competitive superiority of invasive species was exacerbated at high fertility in only one species pair (*S. inaequidens/P. lanceolata*). Some studies have shown an advantage of invasive species in environment with high fertility level especially rich in nitrogen (Brooks 2003, Milchunas and Lauenroth 1995, Hwang et al. 2008). Bradford et al. (2007) found that invasive species did not benefit from elevated N availability at the expense of natives. This was explained by a possible minor response of invasive species to resources manipulations or maybe the species were differentially limited by N. Competitive superiority of the invasive species decreased with fertility in the case of *F. japonica* However, in this case, the limited rooting space in pots may have introduced a bias. Finally, fertility did not alter competitive hierarchy in the case of *S. gigantea*. The response of invasive species to eutrophication is thus highly species dependent.

AQUATIC

We investigated the performance of the invasive Lagarosiphon major and the native Ceratophyllum demersum in monocultures and mixed cultures in relation to sediment dredging (unmanaged versus restored treatment). We mimic these conditions by taking sediments from a) an unmanaged pond with a high amount of organic sediments (unmanaged treatment) and b) a restored pond ecosystem – removal of upper layer – with a lower amount of nutrients in the remnant soil (restored treatment). The objective is to compare growth of L. major and C. demersum both in monocultures and mixed cultures under the two different pond management conditions.

Material and methods

Plants of *C. demersum* and *L. major* were collected in a private pond (N 51° 12.395'; E 4° 40.053') were they co-occur and were kept in 20 °C tap-water for one night before planting. Apical shoot tips, of 10 cm in length, were planted without roots and ramifications into 3 L plastic pots filled with sediment (either pond management condition a or b) to a depth of approximately 5 cm. To avoid soil loss and to stabilize the plants, a 0.5 cm layer of gravel was placed at the top. The pots were placed in 20 L outdoor plastic tanks (30 cm diameter, 31 cm deep). Two-thirds of the aquaria water was replaced each other day to reduce the potential for algal growth and to replenish nutrients in the water column. Sediments and overlying water were collected from the Tenreuken pond, a peri-urban hypereutrophic pond in the Brussels Capital Region were *C. demersum* is the dominant macrophyte (Peretyatko et al. 2007). Monocultures and mixed cultures of *L. major* and *C. demersum* were grown for seven weeks. At the end of week seven, all the plants were harvested, dried at 70 °C for 48 h and the total biomass (shoots + roots) was determined.

To obtain a comprehensive data set, the biomass values from the replicates were grouped together if a statistical comparison of all replicates indicated insignificant differences ($P > 0.05$). The different mean total biomass values were compared using t-tests (restored treatment vs unmanaged treatment; monocultures vs mixtures within a plant species; *L. major* plants vs *C. demersum* plants in monocultures and mixtures). All analyses were performed using STATISTICA v8.

Results

The biomass of the two species calculated after seven weeks in monoculture did not differ significantly, but *L. major* had significantly higher biomass than *C. demersum* in the mixed cultures (unmanaged treatment: t = 9.93, P < 0.001; restored treatment: t = 12.04, P < 0.001, Figure 25). The different pond management conditions influenced the biomass, especially for *L. major*. The biomass of *L. major* plants was significantly higher in the restored treatment both in monocultures (P < 0.05) and mixed cultures (P < 0.001). For *C. demersum* this was only the case in the mixed cultures (Figure 25). A comparison of the biomass between monocultures and mixed cultures indicate that *C. demersum* performed better when growing with a conspecific than a heterospecific neighbour in both pond management conditions (unmanaged treatment: t = 10.15, P < 0.001; restored treatment: t = 6.64, P < 0.001, Figure 25). For *L. major* no significant difference between monocultures and mixed cultures was found.

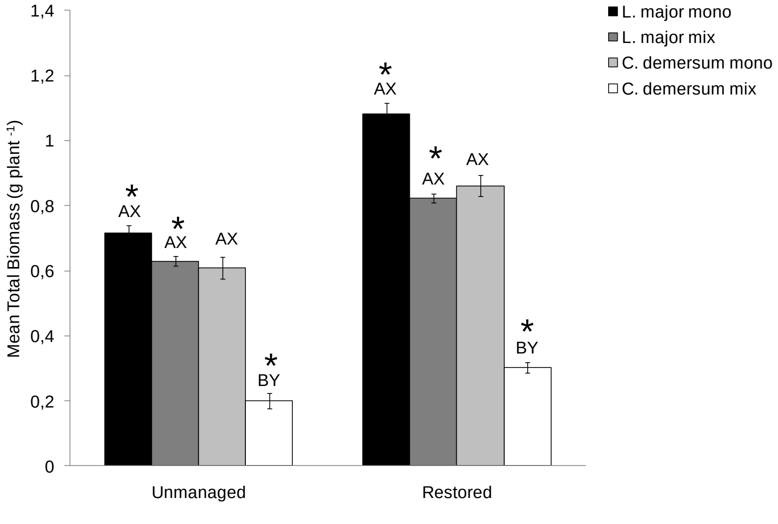


Figure 25: The mean total biomass ± SE of L. major and C. demersum cultivated in monocultures and mixed cultures under two different pond conditions. Statistical differences are indicated by the following symbols: * (L. major plants or C. demersum plants, grown under the two different pond management conditions); A, B (L. major plants or C. demersum plants, grown in monocultures versus mixed cultures); X, Y (L. major plants versus C. demersum plants, grown in monocultures and mixed cultures, respectively).

Discussion

Overall, our results show that *L. major* had a better performance than *C. demersum*, due to a faster growth rate, independent of the characteristics of the growth environment. There was little evidence for interspecific competition (results not shown) so we can ascribe the better performance of *L. major* to a high plasticity under stressful conditions of high pH and low free CO_2 as reported by James et al. (1999). Many eutrophic waters have pH values in the range of 7 to 8, where the concentration of free CO_2 for photosynthetic activity becomes limiting (Kalff 2002). A mechanism that will enable to overcome such limiting conditions would provide an aquatic plant with an advantage in alkaline waters. One such mechanism is the efficient utilization of bicarbonate ions as a dissolved inorganic carbon source that has been described for other Hydrocharitaceae (e.g. *Elodea* sp.) (Prins et al. 1979). It seems that this feature is the key to its success in dominating mixed plant communities as prolonged periods of high pH will suppress the photosynthetic performance of less aggressive submerged macrophytes. When considering both treatments, *L. major* performed better in the restored pond ecosystem, which is expected to be lower in nutrients than unrestored pond ecosystems (Søndergaard et al. 2000). This is in agreement with Rattray et al. (1994) who found that *L. major* had a higher shoot weight when grown in oligotrophic sediment relative to eutrophic sediment. In our case, sediment dredging is not a solution to reduce performance of the invasive *L. major*.

CONCLUSION

For both terrestrial and aquatic invasive species, the results do not support the hypothesis that eutrophication consistently shifts the competitive balance in favour of the invasive species. In terrestrial communities, the trends varied with the studied species. The competitive superiority of the invasive species decreased with fertility in the case of *F. japonica* while it increased for *S. inaequidens*. Eutrophication did not affect the competitive ability of *S. gigantea*. In the aquatic communities, the invasive *L. major* had a better performance than its native competitor, and the growth environment did not modify this.

EFFECTS OF CLIMATE CHANGE ON COMPETITION BETWEEN TERRESTRIAL INVASIVE AND NATIVE SPECIES

Biological invasions and climate warming are two major threats to the world's biodiversity (Ward and Masters 2007). To date, their impacts have largely been considered independently, despite indications that climate warming may increase the success of many invasive alien species (Pyke et al. 2008). Therefore, two experiments were set up to study the responses to warming in native and invasive alien plant species, grown either together or in isolation. In a first experiment, all plants received optimal water to measure only warming effects, while in the second experiment plants received natural precipitation to allow for summer drought. The following questions were addressed: (1) Does climate warming modify the outcome of competition between alien invasive plant species and native competitors? (2) Can the observed patterns be explained by different intrinsic responses of the species to warming?

CLIMATE WARMING WITHOUT SOIL DROUGHT

Materials and methods

Two species pairs were used, each consisting of a HIPS with a native competitor (*S. inaequidens* with *P. lanceolata,* Se-Pl and *S. gigantea* with *E. hirsutum*, So-Ep). The native competitors were selected from the species with highest cover at the field sites of the INPLANBEL project. Monocultures of all four species as well as the two types of mixed communities (HIPS with native competitor in 1:1 ratio) were grown in containers (25 cm diameter, 40 cm deep) and exposed to experimentally induced temperature increase (+3 °C) in climate-controlled greenhouses. Planting density was 18 plants per container. Each type of community was replicated six times in both the unheated and heated treatment.

To detect possible pathways of the warming influence, a combined growth and nitrogen uptake analysis was conducted on each type of monoculture and mixed community. For this purpose, the six replicates per community were harvested at different times during the growing season (July 2007 – September 2007). Plant material was separated into roots and stems. In the mixed communities, invasive and native plants were separated aboveground, which was not possible for the roots. All plant samples were analysed for N concentrations with an NC element analyzer (NC-2100, Carlo

Erba Instruments, Italy). The combined growth and nitrogen uptake analysis involved the calculation of the seasonal time courses of aboveground and belowground biomass (B_{above} and B_{below}), aboveground and belowground nitrogen concentration ($[N]_{above}$ and $[N]_{below}$), total plant biomass (B_{tot}), total plant nitrogen stock (N_{tot}), root specific nitrogen uptake rate (σ_{root}), and total-plant nitrogen use efficiency (NUE) (see Nijs & Impens 1997 for more details). Computing these variables for all species in the monocultures and the mixtures of both the heated and unheated treatment, allows to detect how one species can dominate another in ambient climate, how warming affects the species in monoculture, and how the competitive outcome in mixture is altered by warming. The B_{tot}, N_{tot}, NUE and σ_{root} values of the species could not be calculated in the mixtures as the roots could not be separated.

Nonlinear regression was used to fit the sigmoid models to the variables B_{above}, B_{below}, B_{tot}, N_{above} and N_{tot}. After log-transformation, these variables were further analysed with multifactor analysis of covariance (ANCOVA), with fixed factors origin (native or invasive plant species) and treatment (unheated or heated), and time as a covariate. These analyses were done separately for the species of each pair (if possible), grown either in monoculture or mixture. Differences were considered significant at $p < 0.05$. All statistics were performed using SPSS 16.0 (SPSS Science, Woking, UK).

Results

We first focus on the biomass allocation strategy and the physiology of the species in ambient conditions in monoculture, to understand potential differences in competitive ability. In the first species pair, B_{above} was higher in the alien *S. inaequidens* than in its native counterpart, which was opposite for B_{below} ($F_{1,23} = 5.272$; $p < 0.05$ and $F_{1,23} = 28.408$; $p < 0.05$ for B_{above} and B_{below}, respectively, Figure 26a & b). Together the effects cancelled out in B_{tot} (Figure 26c). Surprisingly, in spite of its smaller root system, *S. inaequidens* acquired more N than *P. lanceolata* ($F_{1,23} = 7.598$, $p < 0.05$, not shown). This was possible owing to a higher σ_{root} in *S. inaequidens* (Figure 26d). *P. lanceolata*, on the other hand, used its acquired N more efficiently (not shown). The two species thus have different biomass allocation strategies and physiological traits, but they reached the same productivity in monoculture in the current climate.

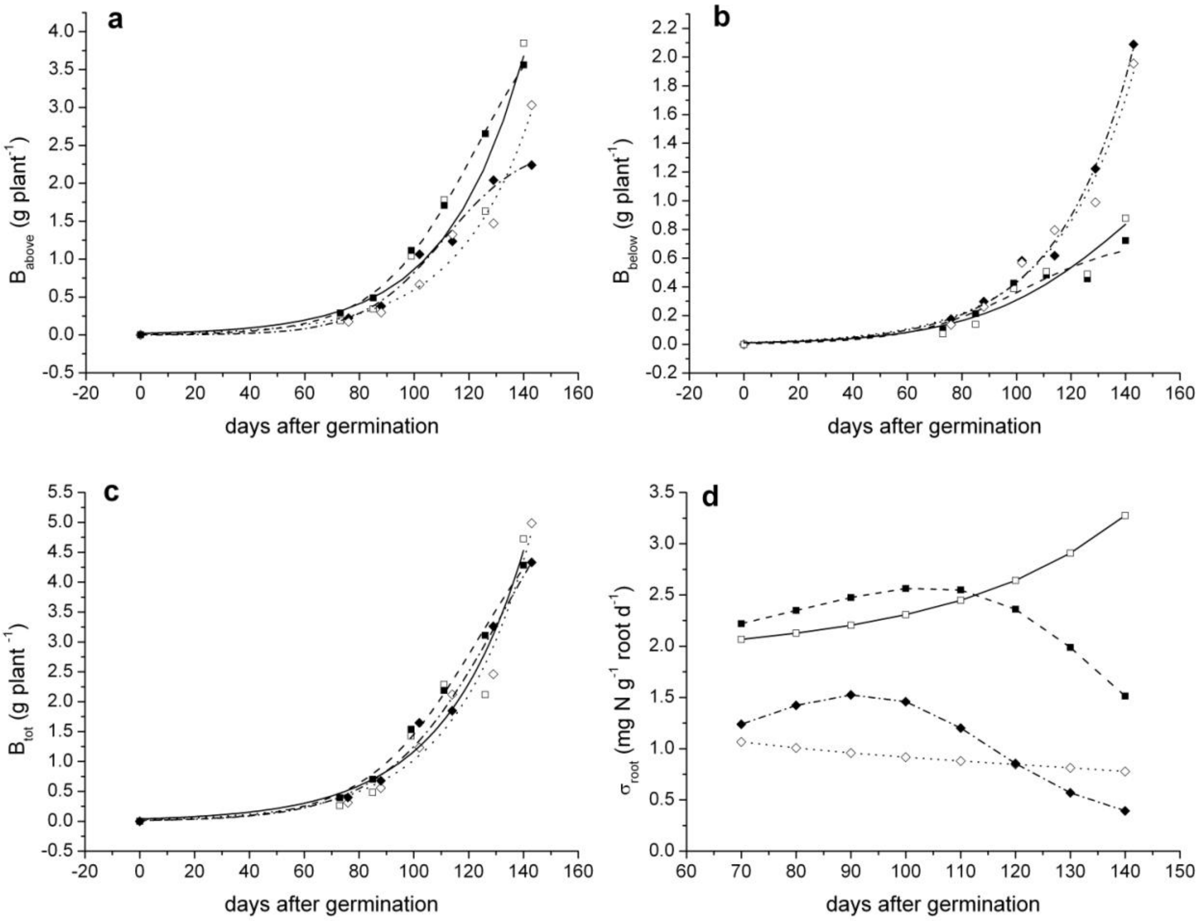


Figure 26: Time courses of aboveground biomass (B_{above}) (**a**), belowground biomass (B_{below}) (**b**), total biomass (B_{tot}) (**c**) and root specific activity (σ_{root}) (**d**) in monocultures of *Senecio inaequidens* grown in unheated (□, __) and heated (+3 °C) (■, _ _) sunlit chambers, and of *Plantago lanceolata* grown in unheated (◇, ...) and heated (+3 °C) (◆, _._.) chambers. (a), (b) and (c): measured values and fitted curves (sigmoidal). (d): curves based on model calculations.

A slightly different pattern was observed in the species of the second pair, the alien *S. gigantea* and the native *E. hirsutum*. Under current climate in monoculture, B_{above} was significantly greater in *E. hirsutum* ($F_{1,23}$ = 6.521, $p < 0.05$, Figure 27a). B_{below}, on the other hand, was similar in the two species (Figure 27 b) and total biomass did not differ significantly either (Figure 27c). *E. hirsutum* tended to acquire more nitrogen than its invasive competitor ($F_{1,23}$ = 4.345, $p = 0.051$, not shown), owing to a higher σ_{root} at the beginning of the season (Figure 27d). This species also exhibited a higher NUE (not shown). The advantage for *E. hirsutum* of a higher σ_{root} disappeared at the end of the season, which may explain why productivity was similar for the two species also in this second pair.

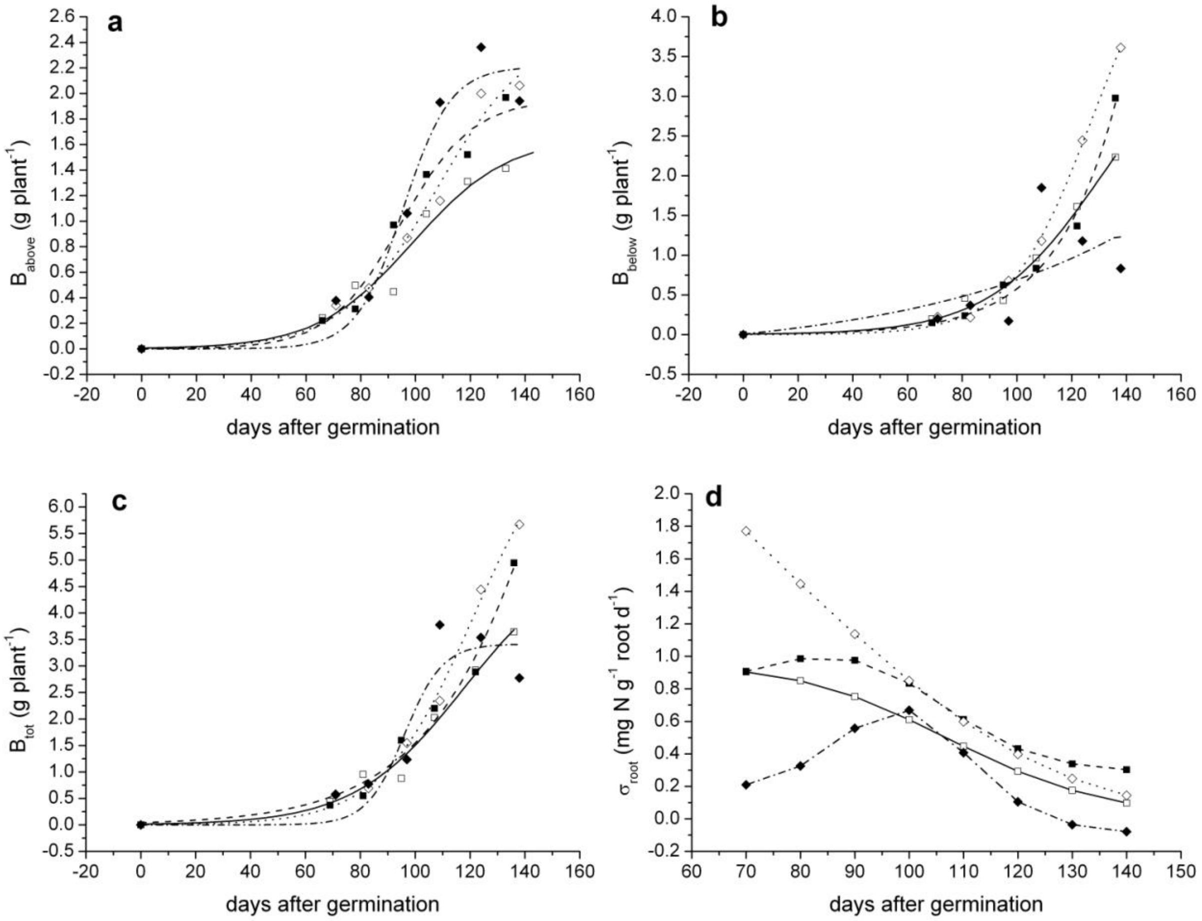


Figure 27: Time courses of aboveground biomass (B_{above}) (**a**), belowground biomass (B_{below}) (**b**), total biomass (B_{tot}) (**c**) root specific activity (σ_{root}) (d) in monocultures of *Solidago gigantea* grown in unheated (□, __) and heated (+3 °C) (■, _ _) sunlit chambers, and monocultures of *Epilobium hirsutum* grown in unheated (◊, ...) and heated (+3 °C) (◆, _._.) chambers. (a), (b) and (c): measured values and fitted curves (sigmoidal). (d): curves based on model calculations.

We next compare the warming influence on these species, still in monoculture to know the intrinsic effect (or lack thereof). Both in *S. inaequidens* and *P. lanceolata*, warming had no significant effect on B_{tot}, B_{above} or B_{below} ($p > 0.05$, Figure 26). Contrary to the first species pair, the warming significantly affected the monocultures of the second pair. In the native *E. hirsutum*, the future climate decreased B_{below} in the second part of the season ($F_{1,5} = 9.356$, $p < 0.05$, Figure 27b). In the invasive *S. gigantea*, on the other hand, warming increased B_{above} in the second part of the growing season ($F_{1,5} = 31.854$, $p < 0.05$, Figure 27a). The root biomass reduction in *E. hirsutum* in the heated chambers later in the season coincided with a decline in root specific activity, whereas root specific activity in *S. gigantea* was stimulated by the warming (Figure 27d).

We conclude that the monocultures of this second pair responded differently to the future climate. The native species suffered from impaired root functioning, while its alien counterpart was favoured.

With the growth strategies of the species and their intrinsic responses to warming analysed, we now turn to the complexity of competing species and ultimately the influence of warming on competition. We first compare monocultures with mixed communities in ambient climate (aboveground, as roots could not be separated). When the species of the first pair were grown together, the alien *S. inaequidens* suppressed the native *P. lanceolata* ($F_{1,11}$ = 7.266, $p < 0.05$, Figure 28a). This was in line with expectations based on the monocultures, where *S. inaequidens* was indeed the most productive (Figure 26a), but growing together apparently amplified this monoculture difference (*P. lanceolata* lost nearly half of its monoculture biomass, *S. inaequidens* gained about one third). How did the simulated warming modify the outcome of competition in this species pair? Warming reduced B_{above} in the alien *S. inaequidens* but increased B_{above} in the native *P. lanceolata*, to the extent that the aboveground advantage of the alien found in ambient conditions by the end of the season had shrunk (Figure 28a). Figure 26d suggests that this biomass convergence between the competitors under warming could originate from σ_{root} values becoming more similar.

When the species of the second pair were grown together in current climate, the native *E. hirsutum* came out on top ($F_{1,7}$ = 7.819, $p < 0.05$; Figure 28b). However, although *E. hirsutum* became about 40% more productive in mixture than in monoculture, *S. gigantea* remained unaffected by its competitor (compare Figure 27a and Figure 28b). Also in this second species pair, the dominant species in mixture had the higher σ_{root} in monoculture (Figure 27d).

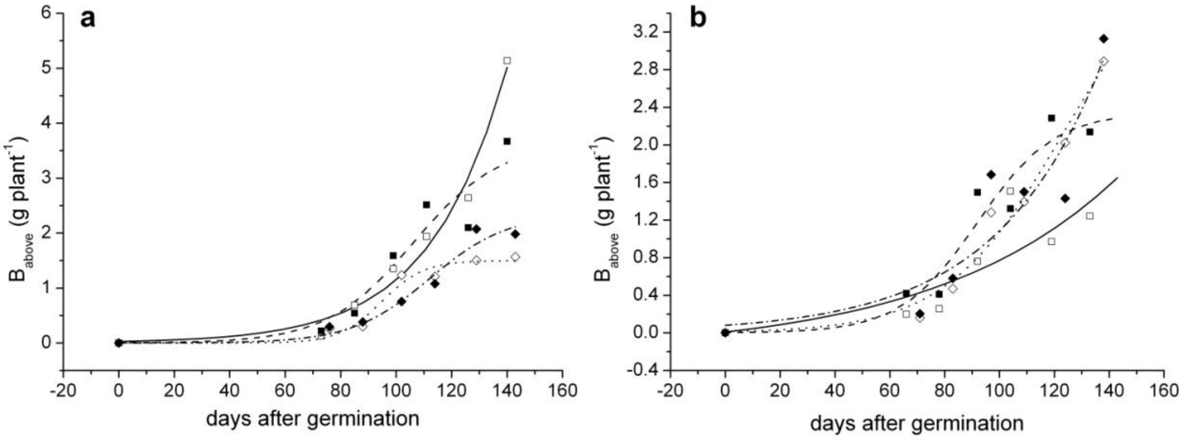


Figure 28: Time courses of aboveground biomass (B_{above}) in mixed communities of species pair *Senecio inaequidens – Plantago lanceolata* (a) and species pair *Solidago gigantea – Epilobium hirsutum* (b). Measured values per plant and fitted curves (sigmoidal) for the alien species of the pair grown in unheated (□, __) and heated (+3 °C) (■, _ _) sunlit chambers, and the native species of the pair grown in unheated (◇, ...) and heated (+3 °C) (◆, _._.) chambers.

Finally, how did warming alter competition between the species of the second pair? In the mixtures, the alien *S. gigantea* was stimulated by the temperature increase ($F_{1,11}$ = 5.191, $p < 0.05$), while the native *E. hirsutum* remained unaffected. As a result, the significant 57% advantage of the native over the alien species observed in mixtures of this pair in ambient conditions (see earlier) was reduced to a non significant 31% in the warmer climate (Figure 28b). The similarity with the first pair is that warming likewise led to convergence of aboveground biomass. The positive warming effect on *S. gigantea* in mixture could again be traced to root specific activity (increased σ_{root} under warming throughout the season, Figure 27d).

Discussion

The ultimate goal of this study was to examine competition between invasive alien species and their native counterparts and the effect of climate warming thereon. Many invasive plant species are strong competitors, and interspecific resource competition is one of the most important processes determining the likelihood of plant invasions (Crawley 1990, Daehler 2003, Vilà and Weiner 2004). Invasive plants can outperform natives by being better at acquiring resources, or more efficient at using them (MacKown et al. 2009, Ren and Zang 2009, Werner et al. 2010). The former is what we observed in the first species pair: given our monoculture data, the alien *S. inaequidens* – known as a very competitive species (López-García and Maillet 2005, Garcia-Serrano et al. 2007) – suppressed the native *P. lanceolata* most likely via greater nutrient uptake rates of its roots. The imbalance between the two species induced by competition was however reduced by the warming, which decreased the aboveground advantage of *S. inaequidens*. Again, the monoculture data suggest that root specific activity was responsible, as this parameter declined in this species in the heated chambers later in the season.

In the mixtures of the second species pair in current climate, the native *E. hirsutum* became more productive than its invasive counterpart, which matched its greater root specific activity. *E. hirsutum* is known as a strong competitor that can indeed crowd out other plant species based on, among other mechanisms, its efficient nutrient uptake (Grime and Mackey 2002). In spite of this, *S. gigantea* remained unaffected by *E. hirsutum*. Possibly these two competiting species occupy different niches leading to stable coexistence (Silvertown 2004). An alternative explanation lies in the fact that *S. gigantea* is known to react with considerable plasticity to interspecific competition by adjusting its morphology and/or physiology (Weber and Jakobs 2005), which may have rendered this species insensitive even to a strong competitor such as *E. hirsutum*. Similar to the first species pair, climate warming modified the competitive balance also in this second pair, by stimulating the suppressed *S. gigantea*. The latter is in line with the greater root nitrogen uptake activity of this species under these conditions observed in the monocultures.

From the examined species pairs, it appears that the sensitivity of the native-invasive interaction to climate warming does not necessarily mirror the intrinsic (monoculture) sensitivities of the species.

CLIMATE WARMING AND ASSOCIATED SOIL DROUGHT

Materials and methods

In this second experiment we investigated the effect of warming and associated changes in water availability on competition. The set-up was similar, but this time, plants receive natural precipitation and an extra species pair was used: *F. japonica* with its native competitor *C. arvense* (Fa-Ci).

Soil water content (SWC) was measured every week during the growing season (from July till September 2008) (PR2 soil moisture sensor, Delta-T Devices Ltd., UK). To detect possible effects of warming and associated soil drought on competition, stress indicators, leaf gas exchange and biomass production were measured on all species, in monocultures and mixed communities, and in both climate treatments. Chlorophyll fluorescence was measured weekly with the Plant Efficiency Analyzer (Hansatech ltd., King's Lynn, Norfolk, England) yielding PSII maximum efficiency (F_v/F_m). To detect underlying causes of possible changes in productivity of the species, Light-saturated photosynthetic rate (P_{max}) and stomatal conductance (g_s) were measured three times during the growing season with a portable gas exchange system (LI-6400, Li-cor, NE, USA). End of September 2008, all plants were harvested, dried for 48 h at 60 °C and weighed. Total biomass (B_{tot}) was separated into below- and aboveground biomass (B_{below} and B_{above}). In the mixed communities, the aboveground plant parts of invasive and native plants were separated, which was not possible belowground.

The effects of climate, chamber (nested within climate), origin (native or alien), culture (monoculture or mixed community, nested within origin), sampling period (for F_v/F_m, P_{max} and gs), and their interactions on all parameters except for SWC were tested with univariate analysis of variance (ANOVA). We used repeated measure analysis of variance (RM-ANOVA) to analyse the effect of climate and chamber (nested within climate) and culture on SWC. Chamber was always treated as random, other factors as fixed. All statistics were performed using SPSS 16.0 (SPSS, Science, Woking UK).

Results

During the entire growing season, average difference in T_{air} between unheated and heated chambers was 3.18 ± (SD) 1.39 °C. This warming led to drier soils in heated communities (RM-ANOVA, $F_{1,49} = 137.211$, $p < 0.001$) with an average SWC of 11.8 ± (SD) 0.6% under ambient temperatures and 8.9 ± (SD) 1.1% under warming.

We first focus on the outcome of competition between invasive plant species and their native competitors in ambient climate. Growing together always modified the aboveground productivity of the species within each pair (significant culture effect, Table IXA), in the sense that the monoculture biomasses diverged in mixture. In species pairs Fa-Ci and So-Ep the native species were favoured, while in Se-Pl the invasive *S. inaequidens* suppressed the native *P. lanceolata* (significant origin effects, Table IX, Figure 29A-C).

Table IX: F-values and significance levels of ANOVAs per species pair (*Fallopia japonica – Cirsium arvense* (Fa – Ci), *Senecio inaequidens – Plantago lanceolata* (Se – Pl) and *Solidago gigantea – Epilobium hirsutum* (So – Ep)) with fixed factors climate, origin and culture (nested within origin) and random factor chamber (nested within climate, not shown) for aboveground biomass (B_{above}) (**A**), with fixed factors climate and origin and random factor chamber (nested within climate, not shown) for B_{above} in mixed communities (**B**) and in monocultures (**C**).

A		climate	origin	climate*origin	culture(origin)	climate* culture(origin)
Fa – Ci	B_{above}	$F_{1,4}$ = 0.597	$F_{1,30}$ = 56.656***	$F_{1,30}$ = 0.271	$F_{2,30}$ = 55.302***	$F_{2,30}$ = 1.233
Se – Pl	B_{above}	$F_{1,40}$ = 14.704***	$F_{1,40}$ = 75.898***	$F_{1,40}$ = 5.716*	$F_{2,40}$ = 6.440**	$F_{2,40}$ = 0.033
So – Ep	B_{above}	$F_{1,40}$ = 5.109*	$F_{1,40}$ = 81.782***	$F_{1,40}$ = 0.451	$F_{2,40}$ = 4.324*	$F_{2,40}$ = 1.252

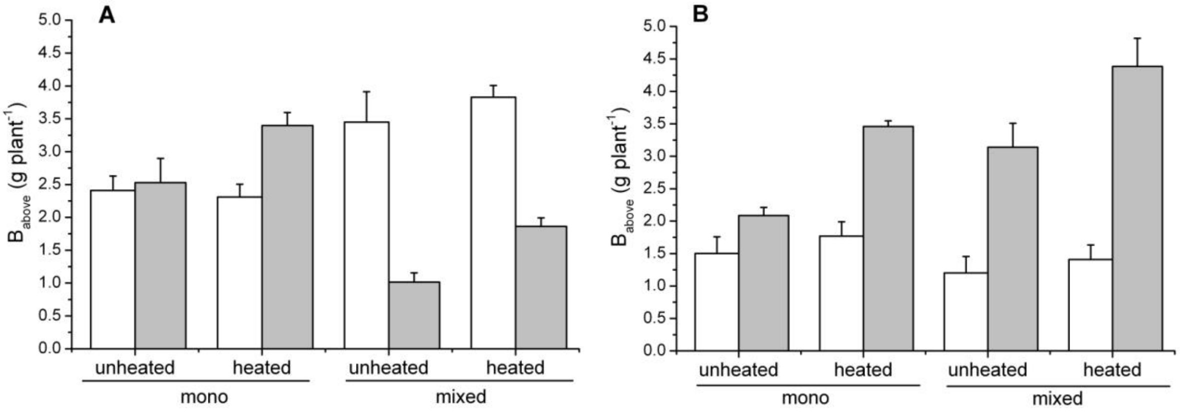
B		climate	origin	climate*origin
Fa – Ci	B_{above}	$F_{1,16}$ = 0.971	$F_{1,16}$ = 124.016***	$F_{1,16}$ = 0.423
Se – Pl	B_{above}	$F_{1,20}$ = 4.556*	$F_{1,20}$ = 46.299***	$F_{1,20}$ = 1.472
So – Ep	B_{above}	$F_{1,20}$ = 3.155	$F_{1,20}$ = 99.315***	$F_{1,20}$ = 3.521

C		climate	origin	climate*origin
Fa – Ci	B_{above}	$F_{1,18}$ = 0.853	$F_{1,18}$ = 3.516	$F_{1,18}$ = 1.858
Se – Pl	B_{above}	$F_{1,20}$ = 17.227***	$F_{1,20}$ = 33.151***	$F_{1,20}$ = 7.889*
So – Ep	B_{above}	$F_{1,20}$ = 2.232	$F_{1,20}$ = 14.730**	$F_{1,20}$ = 0.319

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; otherwise not significant

We subsequently tested whether climate-warming modified these competition outcomes and, if so, whether the intrinsic (monoculture) responses of the species predict such modification. Warming had no effect on B_{above} of the competing species of pair Fa-Ci (Table IX B, Figure 29A), which reflected a similar lack of response in the monocultures (Table IX C, Fig 29A). In the mixtures of pair Se-Pl, on the other hand, warming increased B_{above} in both the native and the invasive species (significant climate effect, Table IXB). Figure 29B indicates a larger increase in *S. inaequidens*, suggesting a trend of climate-warming increasing invader dominance. Those responses again reflected the monocultures, where the native species' B_{above} was insensitive to warming while the invader's B_{above} was increased (ANOVA, $F_{1,10} = 82.404$, $p < 0.05$, Figure 29B). In the third species pair So-Ep, warming did not affect the competitive balance, similar to the first pair (Table IXB). This could again be traced to the monocultures, where none of the biomass parameters responded to climate (Table IXC, Figure 29C).

All species combined, PSII maximum efficiency varied around 0.844 ± (SD) 0.019, which is close to optimal for growing plants (Björkman and Demmig 1987). Warming increased F_v/F_m of all species on average with 1.23% (ANOVA, $F_{1,154} = 25.537$; $F_{1,155} = 11.080$; $F_{1,156} = 74.469$; $p<0.05$ for Fa-Ci, Se-Pl and So-Ep, respectively; not shown), indicating that the soil drought associated with the warming was insufficient to induce severe stress.



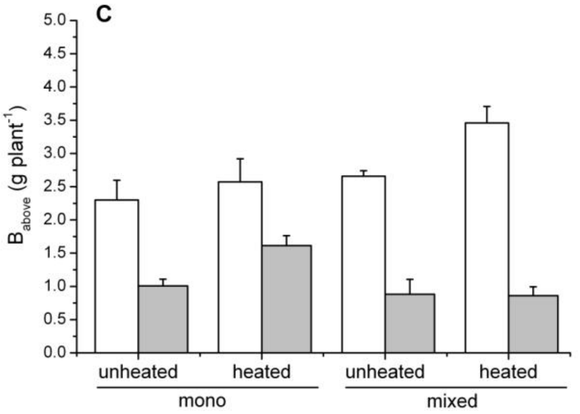


Figure 29: Aboveground biomass (B_{above}) of native (□) and alien invasive (■) plant species, grown in unheated and heated (+3 °C) chambers and in monocultures or mixed communities (1:1 ratio) for species pair *Fallopia japonica – Cirsium arvense* (**A**), *Senecio inaequidens – Plantago lanceolata* (**B**) and *Solidago gigantea – Epilobium hirsutum* (**C**). Mean values ± SE.

Yet the warming did lower g_s in the species of pairs Fa-Ci and So-Ep (ANOVA, $F_{1,116} = 12.996$ and $F_{1,114} = 4.067$, $p < 0.05$ for Fa-Ci and So-Ep, respectively, Figure 30A), which significantly reduced the photosynthetic rates of *F. japonica* and *C. arvense* (ANOVA, $F_{1,116} = 8.291$, $p < 0.05$) but not those of *S. gigantea* and *E. hirsutum* (Figure 30B). In contrast, warming and its associated soil drought had no effect on the stomatal conductance of the species of pair Se-Pl, the only species that became more productive under warming. The photosynthetic rates of *S. inaequidens* were even increased by the warming, opposite to those of *P. lanceolata* which were decreased (ANOVA, significant climate × origin effect, $F_{1,115} = 9.572$, separate ANOVAs per origin, $F_{1,47} = 4.073$, $p < 0.05$; $F_{1,48} = 4.379$, $p < 0.05$ for the native and the alien species, respectively; Figure 30B). This would corroborate the increasing trend in aboveground invader dominance under warming in this pair.

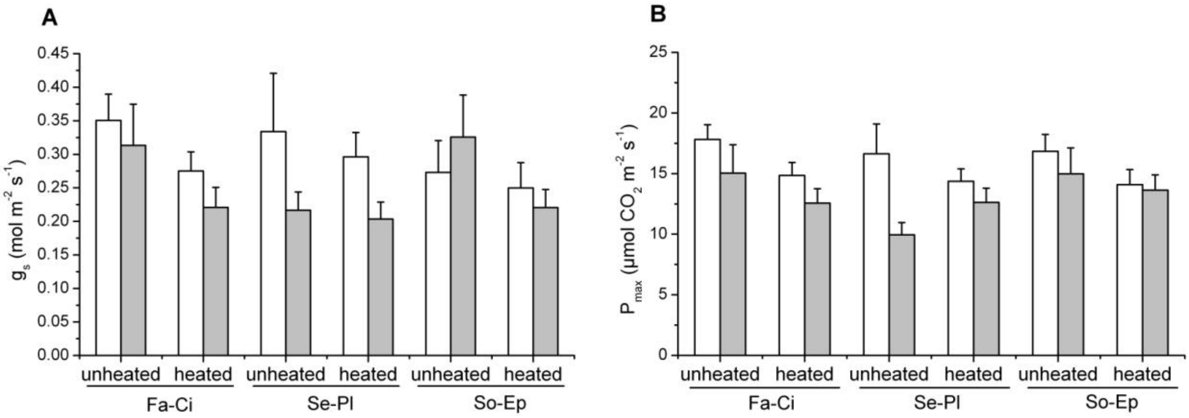


Figure 30: Stomatal conductance (g_s, **A**) and light-saturated photosynthesic rate (P_{max}, **B**) of native (□) and alien invasive (■) plant species in unheated or heated (+3 °C) chambers for species pairs *Fallopia japonica – Cirsium arvense* (Fa – Ci), *Senecio inaequidens – Plantago lanceolata* (Se – Pl) and *Solidago gigantea – Epilobium hirsutum* (So – Ep). Mean values ± SE of three measurement periods (see text for details), two culture types (monocultures and mixed communities) and two measurement temperatures (25 °C and 28 °C).

Discussion

Invasive plants can outperform natives based on traits that promote high growth rate, such as high photosynthetic rates (Baruch and Goldstein 1999, Pysek 2006). Surprisingly, two of the highly invasive plant species examined in the current study were suppressed by their native competitor in ambient climate. In the case of the native *C. arvense* this might be attributed to its higher P_{max} than *F. japonica*; furthermore *C. arvense* too is considered a highly competitive species (Jacobs *et al.* 2006). Similar to *F. japonica*, also *S. gigantea* was suppressed by its native competitor (*E. hirsutum*) in ambient conditions. However, this is in line with the previous experiment, where higher nutrient uptake activities were observed in the latter species compared with *S. gigantea*. The above explains why *C. arvense* and *E. hirsutum* were dominants in the flora of the invaded sites. Possibly, these species represent the upper limit of resistance to the invaders in this study.

Climate warming did not significantly alter the competitive balance in the two aforementioned species pairs. A possible explanation is that both *F. japonica* and *S. gigantea* originate from regions with a temperate climate much similar to that of Belgium, and would consequently benefit less from a warmer environment than invasive alien species with a more southern native range. Furthermore, all four the species of these pairs prefer moist soils (Shamsi and Whitehead 1974, Beerling et al. 1995, Weber and Jacobs 2005, Jacobs et al. 2006,) and where most likely influenced by soil drought as a result of warming (all four species had lower g_s values), but not strongly enough to limit aboveground productivity and not differently enough to modify the competitive balance. In contrast, in the previous experiment, climate warming did affect the competitive balance of So-Ep by stimulating the suppressed invader. These different results could thus potentially be explained by the absence of soil drought in the first experiment.

In the third species pair, the invasive *S. inaequidens* suppressed the native *P. lanceolata* in ambient conditions, which could be expected from its high competitive ability and which was similar to the previous experiment. Warming increased the aboveground biomass of both species, but tended to enhance the invader's dominance. This trend may be explained by the increased photosynthetic rates in the invasive *S. inaequidens* when grown in the heated chambers, as opposed to slightly reduced rates in its native competitor. Also in the previous experiment warming changed the competitive balance of this species pair, but that time by reducing the invader dominance. The native region of *S. inaequidens* (South-Africa) has not only a warmer but also a drier climate, which may explain the different results.

In five of the six species, the biomass responses to warming in the mixed communities could be derived from their intrinsic (i.e. monoculture) responses.

CONCLUSION

In conclusion, simulated climate warming had different effects on the competitive interactions between highly invasive and native species depending on the studied species pair and on the experimental climate conditions. In the experiment where all plants received optimal water supply, climate warming reduced the invader dominance in the pair Se-Ep, but stimulated the suppressed invader in the pair So-Ep. These responses could mostly be traced to σ_{root}. In the experiment where warming was associated with soil drought, the native species dominated in two pairs (Fa-Ci and So-Ep) and climate warming did not modify this competitive balance. In the third pair (Se-Pl), warming tended to increase the dominance of the invader, in agreement with the warmer and drier climate in its native range and with its significantly enhanced photosynthetic rates observed in the experiment.

The observed warming effects on the competitive interactions in these two experiments could in many cases be explained by the intrinsic warming responses of the species.

3. POLICY SUPPORT

HIPS severely endanger species diversity both in terrestrial and aquatic communities, but differences exist which can be useful to guide control

In terrestrial communities, plant species richness loss was a common trend associated with invasion. Especially *Fallopia* spp. and *S. gigantea* exhibited a strong impact on native species richness, starting already at low densities. *I. glandulifera* and *S. inaequidens*, on the other hand, had less impact, except for the latter species at high density.

It would thus be possible to prioritize HIPS for control measures. Impact-density relationships may be used to develop measures adjusted to local invasion status (density). For example, HIPS characterized by steeper curves with more rapidly declining native diversity would require particular attention in the early stages of local expansion. HIPS invade heterogeneous habitats, and, somewhat contrary to expectation, also frequently invade nature reserves. However, in our assessment the presence of HIPS in nature reserves was rather linked to common habitats, characterized by ruderal species. This in any case points to the importance of avoiding disturbance in sites of high biological values to limit nascent foci of invasion. In spite of the severe diversity loss induced by most HIPS, our results did not confirm the generally accepted hypothesis of plant community homogenization, except for *S. gigantea*. Β-diversity thus seems less affected than α-diversity, which suggests that local control measures are more appropriate than landscape design measures.

In aquatic communities, negative impacts on diversity were common as well (for all HIPS), but one group of native species was particularly sensitive: submerged species. This knowledge can help select invaded ponds for control: ponds with those growth forms would require priority. Impact on diversity was again strongly density-dependent, so the same conclusions apply as above.

Impact size was generally of similar magnitude in aquatic and terrestrial HIPS, warranting balanced investment of resources for control.

In order to prevent new invasions, evaluation of the potential eco-risk of newly marketed species (by monitoring or preventive testing if necessary) is needed

Three of the four studied HIPS were introduced as ornamentals. Ornamental horticulture has been recognised as one of the most important pathways for plant invasions. Gardeners select plants that possess characteristics leading to possible invasion, such as a large ecological niche, phenotypic plasticity or easy propagation. For example, in Europe, 57% of the intentionally introduced invasive plants come from horticulture. In nursery gardens, new species from other continents and new hybrids or varieties produced experimentally are constantly increasing. The balsam family is a good example (Adamowski, 2008). *I. glandulifera* and *I. parviflora* introductions in gardens over two centuries have resulted in spectacular invasions in Europe. Nowadays, more than 100 species are marketed, cultivated or used in hybridization tests. Like both invasive balsams, the majority of newly marketed balsam species originate from similar climates to Europe. However, no preventive eco-risk studies are carried out when new horticultural species come onto the market.

Mechanisms of HIPS impact on terrestrial systems: a key role for the soil compartment

Our data provide the first demonstration of a negative impact of *F. japonica* on organic matter cycling. Much of this impact can be ascribed to production of recalcitrant litter (N-poor, lignin-rich), resulting in tight immobilisation of N in the decomposing debris. This may restrict native competitors to grow, even early in the season when they are not yet heavily shaded by *F. japonica*. *F. japonica* can itself apparently overcome this immobilisation through a very high amount of N allocated to aboveground organs and a high N resorption efficiency from senescing shoots. Superior N use and production of recalcitrant litter stand out as two major characteristics of the impacts of *F. japonica* invasion. Both these traits may confer a high competitive superiority. Our data suggest that soil impact may last after *F. japonica* is removed, possibly even requiring topsoil removal to restore previously invaded sites after control.

S. gigantea, on the other hand, affected soil phosphorus pools and fluxes. Lower pH was found in its invaded stands. Soil pH is one of the most important parameters determining adsorption/desorption equilibria of phosphate in soils. We found higher concentrations of bioavailable P in the invaded topsoil, which might be due to higher turnover rates of P in belowground organs and mobilization of soluble P through rhizosphere acidification. Enhanced P avaibility may results in a positive feedback, i.e. aggravation of the competitive superiority of *S. gigantea* over the resident vegetation. Observed modifications in soil P pools may also have an influence on soil biota.

We conclude that HIPS have clear impacts on soils, but use different mechanisms related to different soil elements. Control measures may exploit this information, e.g. liming could be considered in the case of *S. gigantea*.

Impacts of HIPS on the reproductive success of native species

Despite the fact that the HIPS in this study did not have clear negative impacts on the reproductive success of selected native species, our results cannot be generalized to all native and invasive species. A recent quantitative synthesis compiling data from 40 studies revealed an overall negative impact of aliens on visitation rates and reproductive success of native species (Morales and Traveset 2009). Pollinator-mediated impacts of invasive species on natives are a) plant species specific, b) specific to insect category and c) dependent on the degree of fidelity of the latter to one or few plant species. Identifying invasion-sensitive native plant species is thus crucial to improve conservation strategies. More precisely, impacts could be fatal to native plants pollinated by specialist insects if their pollinators are diverted to HIPS.

Effects of HIPS can strongly proliferate to other trophic levels

In terrestrial systems, three of the four studied HIPS exhibited a negative impact on invertebrate density, but *F. japonica* showed the strongest impact. This species induced a dramatic decline in soil fauna abundance under its canopy, so it should be considered as a priority species for control from this perspective. Furthermore, the *F. japonica* impact was greater in open habitat than in closed vegetation, suggesting that open habitat should be given priority. Common to all HIPS was that some taxonomic groups were more affected than others in terms of density. Those groups should be studied in more detail to better understand such impacts. Future studies should also assess impacts at lower taxonomic levels (species) and should examine recovery of faunal assemblage after eradication of the HIPS.

Which factors modify impact?

For the studied aquatic and terrestrial HIPS, our results do not support the hypothesis that high nutrient loading consistently increases the competitive advantage of invasive species relative to native species. Of the four examined species, only the competitive advantage of *S. inaequidens* was enhanced by eutrophication. In many other cases, nutrient reduction may however be important as several studies have shown that native species often can outcompete invasive species in low nutrient conditions (Daehler, 2003 and references therein). However, sediment dredging as a restoration technique is not appropriate to control aquatic invasive plants. Other species pairs (not only submerged species) and other restoration techniques (e.g. nutrient reduction in the water column) should be tested to explain the role of eutrophication in competition and ecosystem invasibility.

Simulated climate warming can modify current competitive interactions between native and invasive terrestrial plants, but the HIPS that we examined did not all respond in the same way. The combination of climate warming and summer soil drought is the most realistic scenario for the future, and in these simulated conditions, the competitive ability of *F. japonioca* and *S. gigantea* was not affected relative to its native counterparts, while the competitive superiority of *S. inaequidens* was increased. The latter species currently exhibits more modest impacts than the other HIPS that we examined, which consequently may change in the future. At the same time, *S. inaequidens* reacted well to eutrophication. These characteristics warrant close surveillance of the future evolution of this species.

The experiments that we did demonstrated that for some species, the climate warming effects on their competitive interaction could be explained or even predicted by the intrinsic warming responses of these species. However, this was not always the case, and further study should investigate which factors are responsible for these differences between the climate sensitivity of species grown in the presence or in the absence of competitors. Screening large numbers of possible mixtures between alien invasive and native species in a future climate may still remain necessary to characterize the future evolution of the threat of invasive alien plant species.

4. DISSEMINATION AND VALORISATION

Science popularisation

Interview I. Nijs on biological invasions in popular scientific magazine Alfabeta (2007, series 21, n° 73, p. 10-15): “Himalayan Balsam and other odd characters” (in Dutch).

Milieufeest / Fête de l’environnement / Brussels Environmental Fair. Participation with info stand, poster and pamflet: “Do you also have exotic species at home?” Brussels, June 1st 2008.

Interview I. Nijs and article in ‘De Morgen’. “Import ban for exotic intruders”. 20 May 2009.

Guided field tour in forests invaded by *Prunus serotina*. Société royale forestière de Belgique. September 2009.

“Invasive alien species, a threat also in Belgium”. Oral presentation and guide (in French). Jardin Massart, Octobre 15th 2009.

“SOS Invasive alien species, a threat also in Belgium”. Oral presentation and guide (in Dutch). Jardin Massart, October 16th 2009.

LIKONA contactday, participation with info stand and poster: “SOS Invasive Aquatic Alien Species in Belgium” (in Dutch). Genk, January 16th 2010.

Nacht van de Onderzoeker / Reseacher’s night, participation with posters, info and experimental stand: “SOS Invasive Aquatic Alien Species in Belgium, Competition between invasive en alien duckweed: een field experiment” (in Dutch). Brussels, September 24th 2010.

Nacht van de Onderzoeker / Reseacher’s night, demonstrations about biodiversity and invasive plant species (in Dutch). Brussels, September 24th 2010.

Scientific contributions

We delivered information to the Belgian Forum on Invasive Species (BFIS, http://ias.biodiversity.be/) concerning impact of *S. gigantea*, *S. inaequidens*, *F. japonica*, *I. glandulifera*, *L. minuta*, *L. major*, *L. grandiflora*, *M. aquaticum* and *H. ranunculoides* on native ecosystems. The BFIS is a permanent web information service organized by the Belgian Biodiversity Platform. It encourages interdisciplinary cooperation among scientists interested in biological invasions and promotes information exchange to support the development of measures dedicated to the prevention and the mitigation of the impacts of invasive species. The BFIS is responsible for preparing and updating the reference list of alien species invading terrestrial, freshwater and marine ecosystems in Belgium, with a focus on organisms causing a strong detrimental impact on native biodiversity.

The GxABT partner and the VUB partner are member of the expert panel of the AlterIAS communication project (LIFE), both concerning aquatic and terrestrial invasive species in Belgium. AlterIAS (Alternatives for Invasive Alien Species) is a communication project which aims at educating the horticultural sector on the invasive plants issue, through raising awareness and preventive measures in order to reduce introductions of those plants in garden, parks, green areas and along roadways, railways and waterways. The project runs throughout Belgium (Wallonia, Flanders, Brussels region).

The VUB partner was member of the expert working group organized by EPPO (European and Mediterranean Plant protection Organization) for a Pest Risk Analysis of *H. ranunculoides*.

The UA partner is member of the “Contact group Invasive Alien Species” of the Federal Ministry of Environment.

The UA partner is member of the advisory board of the Dutch FES programm “Consortium Invasive Plant Species” of the Dutch Ministry of Agriculture and Nature.

Integration paper

An integration paper is almost ready for submission. In this paper the different pathways of impact studied in this project are disentangled for four terrestrial invasive species (*F. japonica*, *S. inaequidens*, *I. glandulifera* and *S. gigantea*) and one aquatic alien species (*L. grandiflora*). Impact is expressed as % change in key variables of the native species or ecosystems, induced by these HIPS.

5. PUBLICATIONS

Peer review

Herr C, Chapuis-Lardy L, Dassonville N, Vanderhoeven S, Meerts P. 2007. Seasonal effect of the exotic invasive plant *Solidago gigantea* on soil phosphorus pools and fluxes. Journal of Plant Nutrition and Soil Science 170: 729-738.

Dassonville N, Vanderhoeven S, Vanparys V, Hayez M, Gruber W, Meerts P. 2008. Impacts of alien invasive plants on soil nutrients are correlated with initial site conditions in NW Europe. Oecologia 157**:** 131-140**.**

Piqueray J, Mahy G, Vanderhoeven S. 2008. Naturalization and impact of a horticultural species, Cotoneaster horizontalis (Rosaceae) in biodiversity hotspots in Belgium. Belgian Journal of Botany 113-124.

Tiébré MS, Saad L, Mahy G. 2008. Landscape dynamics and habitat selection by the alien invasive Fallopia (Polygonaceae) in Belgium. Biodivesity and Conservation 17(10): 2357-2370.

Vanparys V, Meerts P, Jacquemart A-L. 2008. Plant-pollinator interactions: Comparison between an invasive and a native congeneric species. Acta Oecologia 34: 361-369.

Milbau A, Stout JC, Graae BJ, Nijs I. 2009. A hierarchical framework for integrating invasibility experiments incorporating different factors and spatial scales. Biological Invasions 11: 941-950.

Monty A, Mahy G. 2009. Clinal differentiation during invasion: Senecio inaequidens (Asteraceae) along altitudinal gradients in Europe. Oecologia 159 (2): 305-315.

Monty A, Mahy G. 2010. Evolution of dispersal traits along an invasion route in the wind-dispersed Senecio inaequidens (Asteraceae). Oikos 119 (10): 1563-1570.

Njambuya J, Triest L. 2010. Comparative performance between invasive alien *Eichhornia crassipes* and native *Ludwigia stolonifera* under nutrient non-limiting conditions in Lake Naivasha, Kenya. Hydrobiologia 656: 221-231.

Verlinden M, Nijs I. 2010. Alien plant species favoured over congeneric natives under experimental climate warming in temperate Belgian climate. Biological Invasions 12(8): 2777-2787.

Dassonville N, Guillaumaud N, Piola F, Meerts P, Poly F. 2011. The niche construction by the invasive Asian knotweeds (species complex *F. japonica*): impact on activity, abundance and community structure of denitrifiers and nitrifiers. Biological invasions 13 (5): 1115-1133.

Koutika LS, Rainey HJ, Dassonville N. 2011. Impacts of *Solidago gigantea, Prunus serotina, Heracleum mantegazzianum* and *Fallopia japonica* invasions on ecosystems. Applied Ecology and Environmental research 9 (1): 73-83.

Njambuya J, Stiers I, Triest L. 2011. Competition between *Lemna minuta* and *Lemna minor* at different nutrient concentrations. Aquatic Botany 94: 158-164.

Stiers I, Crohain N, Josens G, Triest L. 2011. Impact of three aquatic invasive species on native plants and macroinvertebates in temperate ponds. Biological invasions 13: 2715-2726.

Stiers I, Njambuya J, Triest L. 2011. Competitive abilities of invasive *Lagarosiphon major* and native *Ceratophyllum demersum* in monocultures and mixtures in relation to experimental sediment dredging. Aquatic Botany 95: 161-166.

Vanparys V, Cawoy V, Mahaux O, Jacquemart A-L. 2011. Comparative study of the reproductive ecology of two co-occurring related plant species: the invasive *Senecio inaequidens* and the native *Jacobaea vulgaris*. Plant Ecology and Evolution 144: 3-11.

Vervoort A, Cawoy V, Jacquemart A-L. 2011. Comparative reproductive biology in co-occuring invasive and native *Impatiens* species. International Journal of Plant Sciences 172: 366-377.

Herpigny B., Dassonville N., Ghysels P., Mahy G., Meerts P. 2012. Variation of growth and functional traits of invasive knotweeds (Fallopia spp.) in Belgium. Plant Ecology, in press.

Vervoort A, Jacquemart A-L. Habitat niche sharing between the invasive *Impatiens parviflora* and the native *I. noli-tangere*. Phytocoenologia, in press.

Verlinden M, Nijs I. Effects of climate warming on competition between two highly invasive alien and native plant species explained by root specific nitrogen uptake rate. Submitted.

Verlinden M, Van Kerkhove A, Nijs I. Effects of experimental climate warming and associated soil drought on the competition between three highly invasive West European alien plant species and native counterparts. Submitted.

Others

Vervoort A, Jacquemart A-L. 2007. La problématique des espèces envahissantes. Une approche concrète par l'exemple des balsamines. Forêt wallonne 91 : 10-17.

Vanderhoeven S, Saad L, Tiébré MS, Monty A, Pieret N, Delbart E, Mahy G: Alien invasive species and climate change: overview of research activities. International conference Biodiversity and Climate Change, Belgian Federal Science Policy, Brussels, 21 - 22 May 2007. Poster presentation.

Verlinden M, Nijs I: "Direct responses to temperature increase in alien vs. congeneric native plant species", International conference Biodiversity and Climate Change, Belgian Federal Science Policy, Brussels, 21 - 22 May 2007. Poster presentation.

Vervoort A, Jacquemart A-L. 2007. Comparative pollination study of *Impatiens* species (Balsaminaceae), when a native species faces invasive alien brothers from Asia. 9th International Pollination Symposium on Plant-Pollinator Relationships. Diversity in action, 23–29 July 2007, Ames (Iowa/USA). Poster presentation.

Vanparys V, Jacquemart A-L. Comparison of floral visitors between the invasive *Senecio inaequidens* and the native *S. jacobaea*. 21st meeting of the Scandinavian Association for Pollination Ecologists, Aarhus, DK, 2-4 November 2007. Oral presentation.

Verlinden M, Nijs I: "Influence of climate warming on exotic plants (in Dutch)", Symposium Starters in Nature Research, Brussels, 20 March 2008. Oral presentation.

Jacquemart A-L, Mahaux O, Meerts P, Vanparys V. Comparison of plant-pollinator interactions between an invasive and a native congeneric species, *Senecio inaequidens* and *S. jacobaea*. The evolutionary ecology of plant-animal interactions, Palma de Mallorca, 21-23 April 2008. Poster presentation.

Stiers I and Triest L. The impact of invasive alien plant species on species diversity in aquatic communities. Starters in Natuuronderzoek, March 20th 2008, Brussels, Belgium and Plant Population Biology for the coming decade, 1-3 May 2008, Luxemburg. Poster presentation.

Verlinden M, Nijs I: "Direct responses to temperature increase in alien vs. congeneric native plant species", Biodiversity in an Ecosystem Context, EURECO-GfÖ, Leipzig, 15-19 September 2008. Poster presentation.

Jacquemart A-L, Hans G, Ponette Q & Vervoort A. Existe-t-il un recouvrement de niches entre l'espèce exotique envahissante *Impatiens parviflora* et son congénère indigène, *I. Noli-tangere* ? Colloque "La phytosociologie face aux défis de la connaissance et de la gestion durable des espaces naturels", Velaine-en-Haye, France, 12-14 November 2008. Poster presentation.

Cawoy V, Senterre M, Jacquemart A-L. Pollinator-mediated impacts of the highly invasive *Impatiens glandulifera* on the rare native *Aconitum napellus* subsp. *Lusitanicum*. Insect pollination S.I.G., Royal Entomological Society Rothamsted Research, Harpenden, UK, 3rd December 2008. Poster presentation.

Stiers I and Triest L. Aquatic Invasive Species : Estimating Impact on Biodiversity in Belgium, Themadag Werkgroep Exoten, Rotterdam, December 11th 2008. Hand-outs

Jacquemart A-L, Vervoort A, Vanparys V, Lhoir P, Dassonville N, Saad L, Vanderhoeven S, Mahy G, Cawoy V. 2009. Invasions biologiques : un peu de vocabulaire. Silva Belgica 6: 18-22.

Saad L, Jacquemart A-L, Cawoy V, Vanparys V, Vervoort A, Meerts P, Dassonvile N, Domken S, Mahy G. 2009. Les plantes exotiques envahissantes en Belgique ont-elles des impacts ? Parcs & Réserves 64 (4): 10-16.

Vanderhoeven S, Mahy G, Vanparys V, Jacquemart A-L, Monty A. 2009. Les invasions biologiques ... quand Darwin s'en mêle. Parcs & Réserves 64(4) : 7-9.

Vervoort A, Jacquemart A-L. 2009. La Balsamine à petites fleurs (*Impatiens parviflora*) : une exotique très discrète mais à expansion rapide en forêt. Silva Belgica, 116 (5): 40-43.

Cawoy V, Vanparys V, Jacquemart A-L. Contrasted effects of HIPS presence on the pollination of target native species. ECOVEG 5 : 5ème congrès d'écologie des communautés végétales, Gembloux, Belgium, 8-10 April 2009. Oral presentation.

Domken S, Dassonville N, Josens G, Meerts P. Des changements au niveau des communautés végétales suite aux invasions par *F. Japonica japonica* peuvent affecter les niveaux trophiques supérieurs. ECOVEG 5 : 5ième congrès d'écologie des communautés végétales, Gembloux, Belgium, 8-10 April 2009. Poster presentation.

Saad, L., & Mahy, G. Patterns of impacts of four highly invasive plants species. ECOVEG 5 : 5ième congrès d'écologie des communautés végétales, Gembloux, Belgium, 8-10 April 2009.

Dassonville N, Domken S, Herpigny B, Poly F, Meerts P. Impact of *F. japonica* spp. on ecosystem functioning: Nitrogen and organic matter cycling and implicated soil biota. Science Facing Aliens Conference, Brussels, May 11th 2009. Poster presentation.

Domken S, Dassonville N, Josens G, Meerts P. Impact of *F. japonica japonica* on soil fauna. Science Facing Aliens Conference, Brussels, May 11th 2009. Poster presentation.

Gérard J, Njambuya J, Triest L. Growth performance of invasive alien *Landoltia punctata* and invasive alien *Lemna minuta* in monocultures and mixtures under different nutrient conditions. Science Facing Aliens Conference, Brussels, May 11th 2009. Poster presentation.

Nijs I. ALIEN IMPACT: a project to quantify and explain the biodiversity impacts of highly invasive alien plant species (HIPS). Science Facing Aliens Conference, Brussels, May 11th 2009. Oral presentation.

Nijs I. Taking the heat: will climate warming fuel alien plant invasions and enhance impact on the native flora? Science Facing Aliens Conference, Brussels, May 11th 2009. Oral presentation.

Njambuya J, Triest L. Comparative performance between invasive alien *Eichhornia crassipes* and native *Ludwigia stolonifera* under nutrient non-limiting conditions: Lake Naivasha, Kenya. Science Facing Aliens Conference, Brussels, May 11th 2009. Poster presentation.

Saad L, Jacquemart A-L, Mahy G, Cawoy V. 2009. Plant-pollinator interactions: methodological approaches from the field to an experimental design. Science Facing Aliens Conference, Brussels, May 11th 2009. Oral presentation.

Stiers I, Crohain N, Josens G, Triest L. Impact of aquatic invasive plants on native plant and benthic macro-invertebrate assemblages in Belgian ponds. Science Facing Aliens Conference, Brussels, May 11th 2009. Poster presentation.

Stiers I, Mahy G, Triest L, Saad L. Patterns of impact of HIPS on native vegetation in Belgium. Science Facing Aliens Conference, Brussels, May 11th 2009. Oral presentation.

Vervoort A, Hans G, Ponette Q, Lutts S, Jacquemart AL. 2009. Comparative study of *Impatiens* species (Balsaminaceae). A native species faces invasive alien sisters from Asia Science Facing Aliens, Brussels, 11 May 2009. Poster presentation.

Triest L, Stiers I. Aquatic Invasive Alien Species: Estimating Impact on Biodiversity in Belgium. 1st Symposium on Invasive Aquatic Plant Species in Europe, Düsseldorf, Germany, August 20th 2009. Oral presentation.

Njambuya J, Triest L. Competition between invasive and native *Lemna*: interactive effects of species identity, initial abundance and eutrophication. 2nd Annual Conference Plant Population Biology in a Changing World, Bern, Switzerland, May 21-24 2009 and 12th European Weed Research Society Symposium, Jyväskylä, Finland, August 24-28 2009. Poster presentation.

Stiers I, Crohain N, Josens G, Triest L. Impact of aquatic invasive plants on native plant and benthic macro-invertebrate assemblages in Belgian ponds. Abstract in Proceedings of the 12th European Weed Research Society Symposium, Jyväskylä, Finland, August 24-28 2009. Oral presentation.

Njambuya J, Triest L. Comparative performance between invasive alien *Eichhornia crassipes* and native *Ludwigia stolonifera* under nutrient non-limiting conditions: Lake Naivasha, Kenya. Abstract in Proceedings of the 12th European Weed Research Society Symposium, Jyväskylä, Finland, August 24-28 2009. Oral presentation.

Dassonville N, Domken S, Herpigny B, Poly F, Meerts P. Impact of *F. japonica* spp. on nitrogen cycle and implicated soil biota. World conference on biological invasions and Ecosystem functioning. Porto (Portugal), 27-30 October 2009. Oral presentation.

Domken S, Dassonville N, Josens G, Meerts P. Impact of F. japonica japonica on soil fauna. World conference on biological invasions and Ecosystem functioning. Porto (Portugal), 27-30 October 2009. Poster presentation.

Domken S, Dassonville N, Josens G, Meerts P. Impact of the exotic invasive plant *F. japonica japonica* on soil fauna. Young Botanists' Day, 24-25 November 2009. Oral presentation.

Stiers I, Coussement K, Triest L. Plant-pollinator interactions: showy, invasive *Ludwigia grandiflora* as case study. 6th Neobiota Conference, Copenhagen, Denmark, September 14-17 2010. Poster presentation

Njambuya J, Gérard J, Triest L. Lowering nutrient level might reduce competitive advantage of alien lemnids. 6th Neobiota Conference, Copenhagen, Denmark, September 14-17 2010. Poster presentation.

Gérard J, Njambuya J, Triest L. Relative growth rate and strategy of invasive alien free-floating macrophytes (Lemnids). 6th Neobiota Conference, Copenhagen, Denmark, September 14-17 2010. Poster presentation.

Verlinden M. 2011. Welke exoten winnen bij de klimaatverandering? Eos 9: 9.

6. ACKNOWLEDGEMENTS

ULB would like to thank P. Ghyseels for help with plant and soil analyses and K. Wart and J. Vermander for logistic assistance.

UCL and GxABT would like to acknowledge P. Lhoir and C. Decamps for providing access to the experimental sites, and G. Collignon (RAGR, UCL) for water supplying. Many thanks to Y. Storder (Natagora) for *A. napellum* location information and to D. Jacquemart and L. Saad (GxABT) for *E. angustifolium* populations. We gratefully thank people who helped us to transport the plants, especially the team of GPEX (Service de Gestion du Patrimoine et des Espaces Extérieurs, Université catholique de Louvain), and all the students and members of different labs who took part to the insect observations: E. Affrye, A. Alonso, M. André, Ph. Baret, A. Bauwens, A. Burton, N. Chevalier, M. Descamps, A. Defgnée, M. Detoffoli, L. Dhont, J.-F. Dumasy, B. Franck, C. Gibon, B. Host, A. Jones, G. Kelly, M. Lanotte, J. Lebrun, O. Lehanse, Y. Martin, C. Mayer, S. Meurée, L.-A. Minsart, C. Noel, B. Pernechele, J. Pirotte, M and E. Senterre, J. Tigel-Pourtois, V. Vanparys, F. Vanwindekens, A. Verhegghen and X. Vroman. We thank G. Lognay and C. Marlet from the Department of Analytical Chemistry of AgroBioTech (Gembloux, Liège University, Belgium) for the preliminary scent analyses, A. Vervoort for the preliminary trials with UV patterns, M. Migon for his help in plant cultivation and H. Dailly from CARI (Centre Apicole de Recherche et d'Information, Louvain-la-Neuve) for the chromatography analyses of nectars.

VUB would like to thank Bianca Veraart (province of Antwerp) and the nature managers (Natuurpunt) for providing localities of invaded ponds in the nature reserves. The following landowners generously allowed us to conduct research on their property: M. Farvaque, S. Rogiers, F. De Lathauwer-De Weirdt, W. Orban and J. Henderickx. We thank I. Decordier and the laboratory of Cellular Genetics (VUB) for microscope disposal. We also like to thank the following persons for field/technical assistance: L. Besard, K. Coussement, L. Brans, J. De Weerdt, R. De Schutter, T. Driessens, T. Glorieux, J. Njambuya, T. Sierens, R. Stiers, N. Vanbekbergen, M. Vanderlinden, and A. Van Geert.

Finally, UA thanks F. Kockelbergh and N. Calluy for technical assistance and A. Van kerkhove and J. Hoefnagels for field assistance.

7. REFERENCES

Aarssen LW (1983) Ecological combining ability and competitive combining ability in plants: toward a general evolutionary theory of coexistence in systems of competition. American Naturalist 122: 707-731.

Adamowski W (2008) Balsams on the offensive: the role of planting in the invasion of Impatiens species. In: Tokarska-Guzik B, Brock JH, Brundu G, Child L, Daehler CC, Pyšek P (Eds), Plant invasions: human perception, ecological impacts and management, p. 57–70, Backhuys Publishers, Leiden, The Nederlands, pp. 57-70.

Aguilera AG, Alpert P, Dukes JS and Harrington R (2010) Impacts of the invasive plant F. japonica japonica (Houtt.) on plant communities and ecosystem processes. Biol Invasions 12:1243–1252.

Aizen M A, Morales CL, Morale JM (2008) Invasive mutualists erode native pollination webs. PloS Biology 6(2): 397-403.

Ali MM, Soltan MA (2006) Expansion of *Myriophyllum spicatum* (Eurasian water milfoil) into Lake Nasser, Egypt: Invasive capacity and habitat stability. Aquatic Botany 84: 239-244.

Ashton IW, Hyatt LA, Howe KM, Gurevitch J, Lerdau MT (2005) Invasive species accelerate decomposition and litter nitrogen loss in mixed deciduous forest. Ecological Applications 15, 1263-1272.

Baruch Z, Goldstein G (1999) Leaf construction cost, nutrient concentration, and net CO_2 assimilation of native and invasive species in Hawaii. Oecologia 121:183-192.

Beerling DJ, Huntly B, Bailey JP (1995) Climate and the distribution of *Fallopia japonica*: use of an introduced species to test the predictive capacity of response surfaces. Journal of Vegetation Science 6:269-282.

Beerling DJ, Bailey JP, Conolly AP (1994) *F. japonica japonica* (Houtt.) Ronse Decraene. J Ecol 82:959-979.

Bertrand C, Cochinaire A, Chanut A, Bellvert F, Popovici J, Comte G, Piola F (2008) From allelopathy to agrochemistry: a new approach for the valorisation of invasive plants. Planta Medica 74:1134-1134.

Byers JE (2002) Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. Oikos 97: 449-458.

Bjerknes A-L, Totland Ø, Hegland SJ, Nielsen A (2007) Do alien plant invasions really affect pollination success in native plant species? Biological Conservation 138: 1-12.

Bjorkman O, Demmig B (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170:489-504.

Bradford Ma, Schumacher HB, Catovsky S, Eggers T, Newingtion JE, Tordoff GM (2007) Impacts of invasive plant species onreparian plant assemblages: interactions with elevated atmospheric carbon dioxide and nitrogen deposition. Oecologia 152:791-803.

Brendonck L, Maes J, Rommens W, Dekeza N, Nhiwatiwa T, Barson M, Callebaut V, Phiri C, Moreau K, Gratwicke B, Stevens M, Alyn N, Holsters E, Ollevier F, Marshall B (2003) The impact of water hyacinth (*Eichhornia crassipes*) in a eutrophic subtropical impoundment (Lake Chivero, Zimbabwe). II. Species diversity. Archiv für Hydrobiologie 158: 389-405.

Brooks ML (2003) Effects of increased soil nitrogen on the dominance of alien annual plants in the Mojave Desert. Journal of Applied Ecology 40:344–353.

Crawley MJ (1990) The population dynamics of plants. Philosophical Transactions of the Royal Society B 330(1257):125-140.

Daehler CC (2003) Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restorations. Annual Review of Ecology, Evolution and Systematics 34:183-211.

Dandelot S (2004) Les Ludwigia spp. Invasives du Sud de la France : Historique, Biosystématique, Biologie et Ecologie. Dissertation, Université d'Aix-Marseille III, Marseille.

Dassonville N, Guillaumaud N, Piola F, Meerts P, Poly F (2011) The niche construction by the invasive Asian knotweeds (species complex *F. japonica*): impact on activity, abundance and community structure of denitrifiers and nitrifiers. Biological invasions. In press.

Dassonville N, Vanderhoeven S, Vanparys V, Hayez M, Gruber W, Meerts P (2008) Impacts of alien invasive plants on soil nutrients are correlated with initial site conditions in NW Europe. Oecologia 157: 131-140.

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecolological Monographs 67: 345–366.

Ehrenfeld JG (2006) A potential novel source of information for screening and monitoring the impact of exotic plants on ecosystems. Biological invasions 8(7): 1511-1521.

Garcia-Serrano H, Caňo L, Escarré J, Fleck I, Sans FX (2009) Physiological comparison of alien *Senecio inaequidens* and *S. pterophorus* and native *S. malacitanus*: Implications for invasion. Flora 204:445-455.

Gerber E, Krebs C, Murrell C, Moretti M, Rocklin R, Schaffner U (2008). Exotic invasive knotweeds (F. spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. Biological Conservation 141, 646-654.

Gopal B, Goel U (1993) Competition and allelopathy in aquatic plant communities. Botanical Review 59(3), 155-210.

Grime JP, Mackey JML (2002) The role of plasticity in resource capture by plants. Evolutionary Ecology 16:299-307.

Grabas GP, Laverty TM (1999) The effect of purple loosestrife (*Lythrum salicaria* L.; Lythraceae) on the pollination and reproductive success of sympatric co-flowering wetland plants. Ecoscience 6(2): 230-242.

Hejda M, Pyšek P, Jarošík V (2009) Impact of invasive plants on the species richness, diversity and composition of invaded communities. Journal of Ecology 97: 379–601.

Hejda M, Pyšek P (2006) What is the impact of I. *glandulifera* glandulifera on species diversity of invaded riparian vegetation? Biological Conservation, 132, 143–152.

Hulme PE, Bremner ET (2006) Assessing the impact of Impatiens glandulifera on riparian habitats: partitioning diversity components following species removal. Journal of Applied Ecology, 43, 43–50.

Hussner A (2008) Ökologische und ökophysiologische Charakteristika aquatischer Neophyten in Nordrhein-Westfalen, Dissertation, Heinrich-Heine-Universität, Düsseldorf, Germany.

Hwang BC, Lauenroth WK (2008) Effect of nitrogen, water and igantean density on the growth of *Hesperis matronalis* and two native perennials. Biol Invasions 10(5): 1387-3547.

Jacobs J, Sciengienka J, Menalled F (2006) Ecology and Management of Canada thistle [*Cirsium arvense* (L.) Scop.]. United States Department of Agriculture, Natural Resources Conservation Service, Invasive Species Technical Note 5:1-11.

James CS, Eaton JW, Hardwick K (1999) Competition between three submerged macrophytes, *Elodea* Michx, *Elodea nuttalli* (Planch.) St John and *Lagarosiphon major* (Ridl.) Moss. Hydrobiologia 415: 35-40.

Kalff J (2002) Limnology: inland water ecosystems. Prentice-Hall, Inc., USA.

Kappes H, Lay R and Topp W (2007). Changes in different trophic levels of litter dwelling macrofauna associated with Giant Knotweed Invasion. Ecosystems 119.

Kearns CA, Inouye DW (1993) Techniques for pollination biologists. Colorado, University Press of Colorado.

Kumaga H, Kawai Y, Sawano R, Kurihara H, Yamazaki K, Inoue N (2005) Antimicrobial substances from rhizomes of the giant knotweed *Polygonum sachalinense* against the fish pathogen *Photobacterium danselae* subsp*; piscida*. Zeitschrift für Naturforschung C. A journal of biosciences 60:39-44.

Larson DL, Royer RA, Royer MR (2006) Insect visitation and pollen deposition in an invaded prairie plant community. Biological Conservation 130: 148-159.

Lau JA (2008) Beyond the ecological: Biological invasions alter natural selection on a native plant species. Ecology 89(4): 1023-1031.

Levine JM, Vilà M, D'Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society of London B 270:775-781.

Lindsay EA and French K (2004). *Chrysanthemoides monilifera* ssp *rotundata* invasion alters decomposition rates in coastal areas of south-eastern Australia. For. Ecology and Management198, 387-399.

López-García MC, Maillet J (2005) Biological characteristics of an invasive South African species. Biological Invasions 7:181-194.

Lopezaraiza-Mikel ME, Hayes RB, Whalley MR, Memmott J (2007) The impact of an alien plant on a native plant-pollinator network: an experimental approach. Ecology Letters 10 (7): 539-550.

Mandaville SM (2002) Benthic macroinvertebrates in freshwaters – taxa tolerance values, metrics, and protocols. Project H-1, Soil & Water Conservation Society of Metro Halifax. Xviii, 48 p, Appendices A-B, 120 p.

Mason TJ, French K (2007) Management regimes for a plant invader differentially impact resident communities. Biological conservation 136(2): 246-259.

Maurel N, Salmon S, Ponge JF, Machon N, Moret N and Muratet A (2010) Does the invasive species Reynoutria japonica have an impact on soil and flora in urban wastelands? Biological invasions 61 (0): 1387-3547.

MacKown CT, Jones TA, Johnson DA, Monaco TA, Redinbaugh MG (2009) Nitrogen uptake by perennial and Invasive Annual Grass Seedlings: Nitrogen Form Effects. Soil Science Society of America Journal 73(6):1864-1870.

Memmott J (1999) The structure of a plant-pollinator food web. Ecology Letters, 2: 276–280.

Memmott J, Waser NM (2002) Integration of alien plants into a native flower-pollinator visitation web. Proceedings of the Royal Society of London. Series B, Biological Sciences 269(1508): 2395-2399.

Milchunas DG, Lauenroth WK (1995) Inertia in plant community structure: state of changes after cessation of nutrient-enrichment stress. Ecol Appl 5:452–458.

Moragues E, Traveset A (2005) Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. Biological Conservation 122(4): 611-619.

Morales CL, Traveset A (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. Ecology Letters 12: 716-728.

Nielsen C, Heimes C, Kollmann J (2008) Little evidence for negative effects of an invasive alien plant on pollinator services. Biological Invasions 10(8): 1353-1363.

Nijs I, Impens I (1997) An analysis of the balance between root and shoot activity in *Lolium perenne* cv. Melvina. Effects of CO_2 concentration and air temperature. New Phytologist 135:81-91.

Olff H, Berendse F and De Visser W (1994). Changes in N mineralization, tissue nutrient concentrations and biomass compartmentation after cessation of fertilizer application to mown grassland. Journal of Ecology 82**:** 611–620.

Parepa M, Murrell C,Schaffner U, Bossdorf O (2010). Allelopathic interference between invasive knotweeds and their native competitors. COS 115-8.

Pavela R, Vrchotova N and Sera B (2009). Repellency and toxicity of three Impatiens species (Balsaminaceae) extracts on Myzus persicae Sulzer (Homoptera:Aphididae). Journal of Biopesticides 2(1):48-51.

Peretyatko A, Teissier S, Symoens JJ, Triest L (2007) Phytoplankon biomass and environmental factors over a gradient of clear to turbid peri-urban ponds. Aquatic Conservation: Marine and Freshwater Ecosystems 17: 584-601.

Price MV, Waser NW, Irwin RE, Campbell DR, Brody AK (2005) Temporal and spatial variation in pollination of a montane herb: A seven-year study. Ecology 86: 2106-2116.

Prins HBA, Snel JFH, Helder RJ, Zanstra PE (1979) Photosynthetic bicarbonate utilization in the aquatic angiosperms *Potamogeton* and *Elodea*. Aquatic Ecology 13: 106-111.

Pyke CR, Thomas R, Porter RD, Hellemann JJ, Dukes JS, Lodge DM, Chavarria G (2008) Current practices and future opportunities for policy on climate change and invasive species. Conserv Biol 22:585-592.

Pysek P, Richardson DM (2006) Traits associated with invasiveness in alien plants: Where do we stand? In: Nentwig W, eds. Biological Invasions. Springer, Berlin, pp 97-125.

Pysek P, Richardson DM, Rejmanek M, Webster GL, Williamson M, Kirschner J (2004) Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon 53: 131-143.

Rattray MR, Howard-Williams C, Brown JMA (1994) Rates of early growth of propagules of *Lagarosiphon major* and *Myriophyllum triphyllum* in lakes of differing trophic status. New Zealand Journal of Marine and Freshwater Research 28: 235-241.

Ren MX, Zhang QG (2009) The relative generality of plant invasion mechanisms and predicting future invasive plants. Weed Research 49:449-460.

Ricciardi A, Atkinson SK (2004) Distinctiveness magnifies the impact of biological invaders in aquatic ecosystems. Ecology Letters 7: 781-784.

Richardson DM, Pysek P, Rejmanek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distribution 6: 93-107.

Roubik DW (2001) Ups and downs in pollinator pollinations: when is there a decline? Conservation Ecology, 5.

Scharfy D, Eggenschwiler H, Venterink OH, Edwards PJ and Guesewell S (2009) The invasive alien plant species Solidago igantean alters ecosystem properties across habitats with differing fertility . Journal of Vegetation Science 20(6): 1072-1085.

Scharfy D, Güsewell S, Gessner MO and Venterink H (2010) Invasion of Solidago gigantea in contrasting experimental plant communities: effects on soil microbes, nutrients and plant–soil feedbacks. Journal of Ecology 98(6): 1379-1388.

Shamsi SRA, Whitehead FH (1974) Comparative ecophysiology of *Epilobium hirsutum* L. and *Lythrum salicaria* L. 1. General biology, distribution and germination. Journal of Ecology 62(1):279-290.

Silvertown J (2004) Plant coexistence and the niche. Trends in Ecology and Evolution 19(11):605-611.

Skubaa P, Mierny A (2009) Invasive *Reynoutria* taxa as a contaminant of soil. Does it reduce abundance and diversity of microarthropods and damage soil habitat? Pestycydy 1 : 57-62.

Søndergaard M, Jeppesen E, Peder Jensen J, Lauridsen T (2000) Lake restoration in Denmark. Lake Reservoir Management 5: 151-159.

Subbarao GV, Rondon M, Ito O, Ishikawa T, Rao IM, Nakahara K, Lascano C, Berry WL (2007) Biological nitrification inhibition (BNI)_Is it a widespread phenomenon ? Plant Soil 294:5-18.

Tateno M, Hirose T (1987) Nitrification and nitrogen accumulation in the early stages of primary succession on Mt. Fuji. Ecol Res 2:113-120.

Vilà M, Bartomeus I, Dietzsch AC, Petanidou T, Steffan-Dewenter I, Stout JC, Tscheulin T (2009) Invasive plant integration into native plant–pollinator networks across Europe. Proceedings of the Royal Society of Botany 276: 3887-3893.

Vilà M, Weiner J (2004) Are invasive plant species better competitors than native plant species? – evidence from pair-wise experiments. Oikos 105:229-238.

Ward NL, Masters GJ (2007) Linking climate change and species invasions: an illustration using insect herbivores. Global Change Biology 13:1605-1615.

Weber E, Jakobs G (2005) Biological flora of central Europe: *Solidago gigantea* Aiton. Flora 200:109-118.

Werner C, Zumkier U, Beyschlag W, Máguas C (2010) High competitiveness of a resource demanding invasive acacia under low resource supply. Plant Ecology 206: 83-96.

Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiol Mol Rev 61:533–569.