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,
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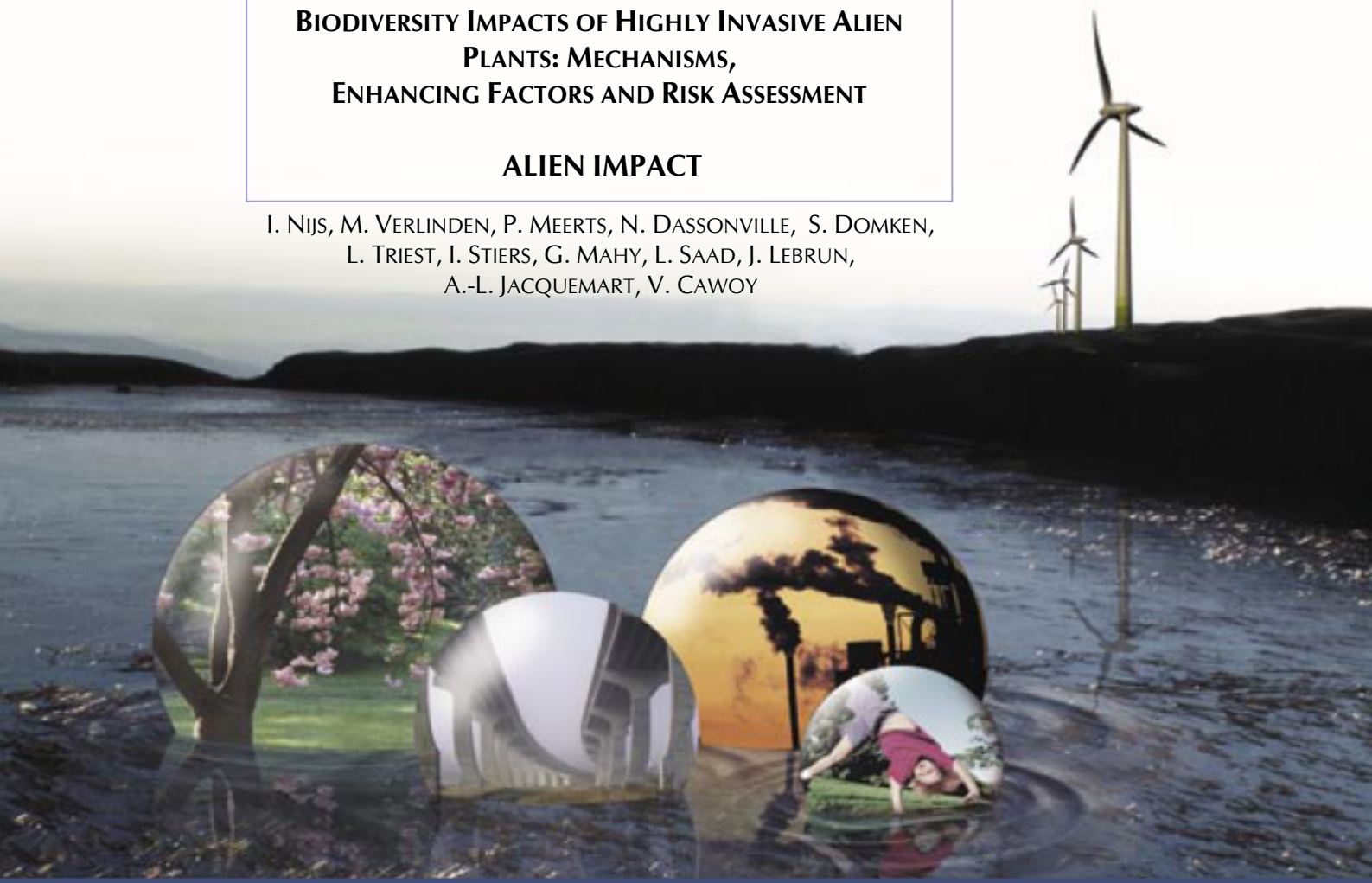

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SCIENCE FOR A SUSTAINABLE DEVELOPMENT (SSD)

Biodiversity

FINAL REPORT PHASE 1

BIODIVERSITY IMPACTS OF HIGHLY INVASIVE ALIEN PLANTS: MECHANISMS, ENHANCING FACTORS AND RISK ASSESSMENT

ALIEN IMPACT

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ACRONYMS, ABBREVIATIONS AND UNITS

Ø	diameter
σ_{root}	specific root activity
AGR	absolute growth rate
ANCOVA	analysis of covariance
ANOVA	analysis of variance
APHA-AWWA-WEF	American Public Health Association - American Water Works Association - Water Environment Federation
B_{above}	aboveground biomass
B_{below}	belowground biomass
B_{tot}	whole-plant biomass
Bicarb-Pi	inorganic phopsphorus extracted with bicarbonate
C	carbon
CANOCO	canonical community ordination
CCA	canonical correspondence analysis
Chl a	chlorophyll a
df	degrees of freedom
DIN	dissolved inorganic nitrogen
FUSAGx	Faculté universitaire des sciences agronomiques de Gembloux
gamma r	gamma correlation coefficient
HIPS	highly invasive plant species
INPLANBEL	Invasive plants in Belgium: patterns, processes and monitoring (project)
JI	Jaccard Index (similarity coefficient)
MS	mean square
n	number of replicates
N	nitrogen
$[N]_{above}$	aboveground nitrogen concentration
$[N]_{below}$	belowground nitrogen concentration
$[N]_{tot}$	whole-plant nitrogen concentration
NaOH-Pi	inorganic phosphorus extracted with sodium hydroxide
NH_4^+	ammonium
NO_2^-	nitrite
NO_3^-	nitrate
NUE	nitrogen use efficiency
p	value of significance
P	phosphorus
PCA	principal components analysis
pH	degree of acidity
PME	phosphomonoesterase
PS	proportional similarity
Resin-Pi	inorganic phosphorus extracted with anion exchange resin
RGR	relative growth rate
RGRD	relative growth rate difference
r_s	spearman rank correlation coefficient
SD	standard deviation
SGIB	Sites de Grand Intérêt Biologiques
spp.	species
SRP	soluble reactive phosphorus
TP	total phosphorus
UA	Universiteit Antwerpen
UCL	Université Catholique de Louvain
ULB	Université Libre de Bruxelles
VUB	Vrije Universiteit Brussel

1. SUMMARY

Introduction and goals

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. Developing effective prevention stategies 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 ALIEN IMPACT proposal aims to provide a first integrated study of patterns and mechanisms of impact by alien invasive species in Belgium. It considers multiple, highly invasive plant species (HIPS), and combines 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 are studied. The main objectives are:

(1) Identify the **effect of HIPS on the diversity of native plant communities**, characterizing communities that experience greatest impact and characterizing sensitive species that might serve as bio-indicators for impact. Analyze whether critical invader densities exist above which impacts enhance disproportionately, and whether low densities can already induce high impact.

(2) Identify **mechanisms of HIPS impact on native plants**, and quantitatively disentangle the pathways involved through manipulation experiments:
- Are effects of HIPS mediated primarily by competition for soil resources, by competition for pollinator resources, or by other mechanisms such as secretion of allelochemicals?
- Does modification of ecosystem properties (soil) triggered by HIPS reinforce impact on native plant species?

(3) **Impacts at other trophic levels**: Assess whether HIPS impact on native plant diversity is associated with diversity loss or changes in community structure in other trophic groups, notably soil fauna (bacterivores, fungivores, detrivores, omnivores/predators, ...) and macro-invertebrates in water and sediment. Are such changes mediated by modification of ecosystem properties?

(4) Analyse **factors that may modify HIPS impacts on native plant species** in the future:
- Does eutrophication aggravate impacts of invasion by modifying the outcome of HIPS competition with native species?
- Does climate warming alter HIPS impact on native species?

Partner 1 (UA) studies direct mechanisms of impact on native terrestrial plants via competition and how climate warming modifies this. Partner 2 (ULB) investigates indirect

mechanisms of impact on native plants via soil modification, impact on soil fauna, and how soil eutrophication influences impact (all terrestrial). Partner 3 (VUB) focuses on impact on aquatic plant communities and associated other trophic levels, and on how eutrophication of water will modify impact. Partner 4 (FUSAGx) studies patterns of impact on native terrestrial plant communities at larger scale (up to landscape) and, together with partner 5 (UCL), indirect mechanisms of impact on terrestrial plants via pollination. To integrate these divergent types of information, a twofold strategy was adopted:
(1) Partners work with a common set of species for terrestrial plants, and organize the field work in the same landscape/sites when relevant. For most studies, there is an aquatic counterpart to allow comparison with terrestrial invaders. Experimental protocols are shared when possible.
(2) Data from different experiments are combined in additional, integrated analysis.

This report of phase 1 presents results for the majority of the above-mentioned studies, together with preliminary conclusions and recommendations.

Effect of HIPS on the diversity of native plant communities

Concerning impacts on biodiversity, the study that tested whether species diversity was affected by HIPS in terrestrial communities, found a reduction in mean species richness of the native vegetation following invasion by three target HIPS: *Fallopia* spp., *Impatiens glandulifera* and *Solidago gigantea*. There was no plant species richness loss associated with invasion for *Senecio inaequidens*. Impact increased with HIPS density except for *I. glandulifera*. It would thus be possible to prioritize HIPS for control measures depending on species. HIPS invade heterogeneous habitats, and, somewhat contrary to expectation, also frequently invade nature reserves (though not necessarily the vulnerable habitats). In spite of the severe diversity loss induced by most HIPS, our results did not confirm the generally accepted hypothesis of plant communities homogenisation, except for *Solidago gigantea*.

The aquatic counterpart study revealed that the presence of all studied HIPS (*Ludwigia grandiflora*, *Hydrocotyle ranunculoides* and *Myriophyllum aquaticum*) negatively affected both submerged and emergent native vegetation, but the submerged vegetation had significantly lower cover with increasing HIPS cover. This knowledge can help select invaded ponds for control: ponds with those growth forms may require priority. Multivariate analysis revealed a pollution gradient, with *H. ranunculoides* at higher eutrophication levels than *L. grandiflora* or *M. aquaticum*.

We conlude that HIPS can severely endanger species diversity both in terrestrial and aquatic communities, but differences exist, which can be exploited to guide control.

Mechanisms of HIPS impact on native plants

A study on indirect impacts by HIPS via pollinators in terrestrial systems investigated whether HIPS affect reproductive success of native plant species and whether those impacts are mediated by modification of pollinator services. For two HIPS, *I. glandulifera* and *S. inaequidens*, native counterparts were found that share similar habitats and insect visitors and have overlapping flowering periods. From a controlled experiment with three species pairs (*I. glandulifera - E. angustifolium*, *I. glandulifera - A. napellus* and *S. inaequidens – S. jacobaea*) it was clear that pollinator-mediated impacts of HIPS on native species were specific to each pair. For *S. inaequidens – S. jacobaea*, only a slight negative impact on the number of flowers visited per trip by an insect was detected. This change in the insect

foraging behavior had no impact on the reproductive success of *S. jacobaea*. The presence of *I. glandulifera*, on the other hand, influenced the pollinator guild of *E. angustifolium* and *A. napellus* by highly increasing the proportional similarity between native – invasive. Despite this modification in pollinator guild, *I. glandulifera* had no impact on the reproductive success of *A. napellus*, while it had a global facilitative impact on *E. angustifolum*. The first results thus indicate that the observed negative impacts of the HIPS on native cover may not be realized via this indirect pathway.

Two experiments that examined underlying mechanisms of HIPS impact on native terrestrial plants via soil modification have been conducted. One study investigated organic matter and nitrogen cycling in *F. japonica* and native competitors. Litter of native species decomposed four times faster than litter of *F. japonica*, which could be explained by initial differences in chemical composition. The invasion of *F. japonica* also seems to influence decomposition by modifying micro-environmental conditions. Concerning N dynamics, immobilization occurs in *F. japonica* litter, while the species has an efficient resorption in belowground organs. Another experiment studied impacts of *S. gigantea* on phosphorus. Labile P pools were always higher and soil pH always lower in invaded stands. Soil pH is one of the most important parameters determining adsorption/desorption equilibria of phosphate in soils. Also the concentrations of bioavailable P were higher 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. The observed increase in P pools of belowground organs is due to both increased biomass and increased P concentrations in invaded plots. The highly invasive *S. gigantea* thus clearly alters the phosphorus cycle in native ecosystems. 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*.

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. However, both in invaded and uninvaded soil, the native competitor *C. arvense* grew better in pure culture in the absence of charcoal 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

Regarding impacts at other trophic levels, we found an influence of *F. japonica* on soil fauna assemblages. Although soil fauna diversity was not affected, soil fauna density strongly declined under *F. japonica*: the total number of individuals was 50 tot 60% lower in invaded plots. This decrease might be explained by reduced habitat heterogeneity and resource diversity in *F. japonica* stands. Altered microclimatic conditions might, in part, explain shifts in faunal assemblages. Data analysis about the effect of *S. gigantea* on soil fauna will be accomplished in phase 2.

In aquatic systems, the presence of alien plants and their detritus appears to have a negative impact on the structure of the macro-invertebrate community. Ponds invaded with *L. grandiflora* suffered the most in terms of loss of both macro-invertebrate abundance and diversity. Later identification of phytoplankton and zooplankton will allow

us to verify whether effects can be generalised to more trophic levels in both water and detritus.

On the basis of this partial assessment, we conclude that effects of HIPS can strongly proliferate to other trophic levels.

Factors that may modify HIPS impacts on native plant species

Research on factors that may modify HIPS impact on native plants focused on the effects of eutrophication and climate warming on competition between invasive and native plant species.

Data to characterize a possible enhancing or mitigating influence of eutrophication on impact will be provided in phase 2 of the project. A preparatory experiment for aquatic systems showed the competitive superiority of the invasive *L. minuta* over the native *L. minor*. Model analysis showed this was due to *L. minuta*'s higher relative growth rate. The next step is to assess whether different nutrient levels will affect competition in this system.

Simulated climate warming significantly modified current competitive interactions between native and invasive terrestrial plants. However, the way in which the balance between HIPS and native species was altered, depended on the studied species pair. In the current climate, in one pair (*S. inaequidens – P. lanceolata*) the HIPS dominated, and in the other pair (*S. gigantea – E. hirsutum*) the native species. Climate warming reduced the HIPS dominance in the first species pair, but stimulated the supressed HIPS in the second. Most of these changes could be ascribed to warming influences on root specific nitrogen uptake capacity, again confirming the importance of belowground processes. From the species pairs examined, it appears that the sensitivity of the native-invasive interaction to climate warming does not necessarily mirror the intrinsic (monoculture) sensitivities of the species. This would imply that predicting the outcome may be complex. In a second experiment, the influence of higher temperatures and associated changes in water availability on competition between native and invasive species was investigated (data analysis in progress).

Conclusions and phase 2 prospects

Our results up to now support that HIPS do more to ecosystems than merely suppress native competitors. There are strong indications for a wide range of HIPS impacts, both in terrestrial and aquatic systems, in magnitude and pathway, and at different scales and levels of biological organisation (individual, population, community, ecosystem). A number of these impacts are severe. Surprisingly, impacts higher up in the foodweb are not necessarily weak, and the soil is a key compartment for understanding impacts. The data also provide experimental support for the widespread hypothesis that climate change is likely to alter alien plant invasions.

During phase two, we will finish data analysis for some of the above-mentioned studies and we will investigate observed effects in more detail. On the other hand, several new experiments will be conducted. Regarding mechanisms of HIPS impact on native plants, a field experiment will be conducted to assess whether niche overlap can explain the outcome of terrestrial native-invasive competition at microscale. A similar experiment about indirect impacts of HIPS on native plants mediated by pollinators as done for terrestrial plants will also be conducted for aquatic systems. In addition, a study to

investigate the effects of eutrophication on competition between native and invasive species in terrestrial systems will start.

When the experimental work is phasing out, we will conduct combined analyses on data from different work packages to disentangle impact pathways and compare impact at different spatial scales.

2. INTRODUCTION

2.1 Context

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).
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).

2.2 Objectives

The project aims to provide a first integrated study of patterns and mechanisms of impact by alien invasive species in Belgium. It will consider different spatial scales and multiple levels of ecological organisation. The project will consider both terrestrial and fresh water ecosystems. Its central aim is impact on biodiversity. We will focus 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 will be studied. The project will concentrate on highly invasive plant species (HIPS) 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 is to reconcile these conflicting prerequisites in a single study.

2.3 Expected outcomes

The results of the project are expected to create spinoffs useful for ecosystem management and restoration. Sensitive species might be identified that could serve as bio-indicators for impact. Knowledge on carry-over effects of HIPS, mediated by modification of soil properties, can be used to estimate the probability of successful restoration of infested sites. Regional administrations for environment and agriculture can take into account estimates of aggravation of invasion impact by eutrophication, for decisions on the sustainability of agricultural practise. Action plans to mitigate effects of climate change can take into account expected changes in the impacts of invasive plant species through climate change. Comparison of impact between terrestrial and aquatic systems could aid policy makers in prioritisation of means. The knowledge on HIPS impact acquired in the project will also be used to adjust the Black List and Watch List of exotic species of the Belgian Biodiversity Platform. The results of the project will be actively disseminated to the stakeholders, including via specific activities to raise awareness. These include demonstrations in existing experimental gardens, a brochure on aquatic invasion impact, and guided field tours to in situ experience impact of HIPS on native biodiversity. The results will also be submitted for publication to leading journals in ecology.

3. REPORT OF THE PROGRESS AND IMPLEMENTATION OF THE METHODOLOGY

WP 1 PATTERNS OF IMPACT OF HIPS ON NATIVE PLANT COMMUNITIES

Task 1.1 Is species diversity affected by HIPS in terrestrial communities? (FUSAGx, month 1-21; UA month 6-7)

In this task we aim to 1) test whether HIPS colonize habitats of high biological value in Belgium; 2) test whether HIPS affect local native species richness and whether the impact is related to HIPS density; 3) test whether HIPS invasion leads to vegetation homogenisation.
This study was conducted throughout Belgium. Sites were chosen in geographically distinct areas and covered a range of habitats. Common sites such as road sides or ruderal places were included but a particular focus was put on sites of high biological value (nature reserves, Natura 2000, SGIB), where impact on habitat of high biological value could be expected. Considering both HIPS population size as a limiting factor (very small populations could not be studied) and the need to cover as much as possible the heterogeneity of invaded habitats, several sites were visited before selection for each HIPS: *Fallopia* spp. (n visited = 22; n selected = 10), *S. inaequidens* (n visited = 26; n selected = 10), *I. glandulifera* (n visited = 29; n selected = 11), *Solidago* spp. (n visited = 25; n selected = 11). Note that only *S. gigantea* was considered in our study as only one site harbouring a few clumps of *S. canadensis* was found. At each selected site (Table 1), 12 quadrats (six in invaded and six in uninvaded vegetation) were taken, including a gradient of increasing HIPS density. Within each quadrat, the percentage cover of all plant species was recorded.

Table 1: Selected study sites for the 4 target HIPS, protection status, and type of habitat.

HIPS	Site code	Site protection status	Habitat
Fallopia x bohemica	RixCon	SGIB	Riparian forest
Fallopia japonica	BouSeb	SGIB	Riparian forest
Fallopia japonica +F. x bohemica	BlaBru	Nature reserve	Sand pit
Fallopia japonica	FreCar	SGIB	Meadow
Fallopia japonica	SavMil	/	Road side
Fallopia japonica	KalMee	Nature reserve	Meadow
Fallopia x bohemica	TerDoe	Nature reserve	River bank
Fallopia japonica	HobPlo	Nature reserve	Park
Fallopia japonica	ComTar	Nature reserve	Road side
Fallopia japonica	OlRou	/	Road side
Senecio inaequidens	GemSab	SGIB	Sand pit
Senecio inaequidens	AcoGar	SGIB	Abandoned railway
Senecio inaequidens	FloCar	SGIB	Quarry
Senecio inaequidens	RocLam	SGIB	Rocky cliff
Senecio inaequidens	SpoCar	SGIB	Quarry
Senecio inaequidens	BlaBru	Nature reserve	Sand pit
Senecio inaequidens	SchGar	/	Dump
Senecio inaequidens	ComCar	SGIB	Rocky cliff
Senecio inaequidens	ArcSab	SGIB	Sand pit
Senecio inaequidens	ColTer	/	Coal tip
Impatiens glandulifera	DouVlr	/	River bank
Impatiens glandulifera	LanFra	SGIB	Spawning area
Impatiens glandulifera	ItrSen	SGIB	River bank
Impatiens glandulifera	DurOur	SGIB	Ruderal humid zone
Impatiens glandulifera	LauBoi	/	Forest understorey
Impatiens glandulifera	DinCol	SGIB	River bank
Impatiens glandulifera	HowEln	/	River bank
Impatiens glandulifera	HauSab	Close to SGIB	Forest understorey
Impatiens glandulifera	FraNou	SGIB	River bank
Impatiens glandulifera	ComOur	/	River bank
Impatiens glandulifera	WicMeg	/	Moist meadow
Solidago gigantea	BauRou	/	Road side
Solidago gigantea	LibRou	/	Green wastes

Solidago gigantea	HauSab	SGIB	Sand pit
Solidago gigantea	BraSab	SGIB	Sand pit / side of road
Solidago gigantea	MabRou	/	Road side
Solidago gigantea	FicCar	SGIB	Quarry
Solidago gigantea	TilRou	/	Road side
Solidago gigantea	LonMeg	SGIB	Moist meadow
Solidago gigantea	ReiGar	/	Railway
Solidago gigantea	TheRou	/	Road side
Solidago gigantea	RixHum	Natural reserve	Moist meadow

Invaded vegetation types largely overlapped between *Fallopia* spp., *S. gigantea* and *I. glandulifera*, while vegetation invaded by *S. inaequidens* was characterized by a different floristic composition (Fig. 1). 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 critical habitat (Natura 2000 habitat). No native species with patrimonial value (red list) were found in the releves of the uninvaded vegetation.

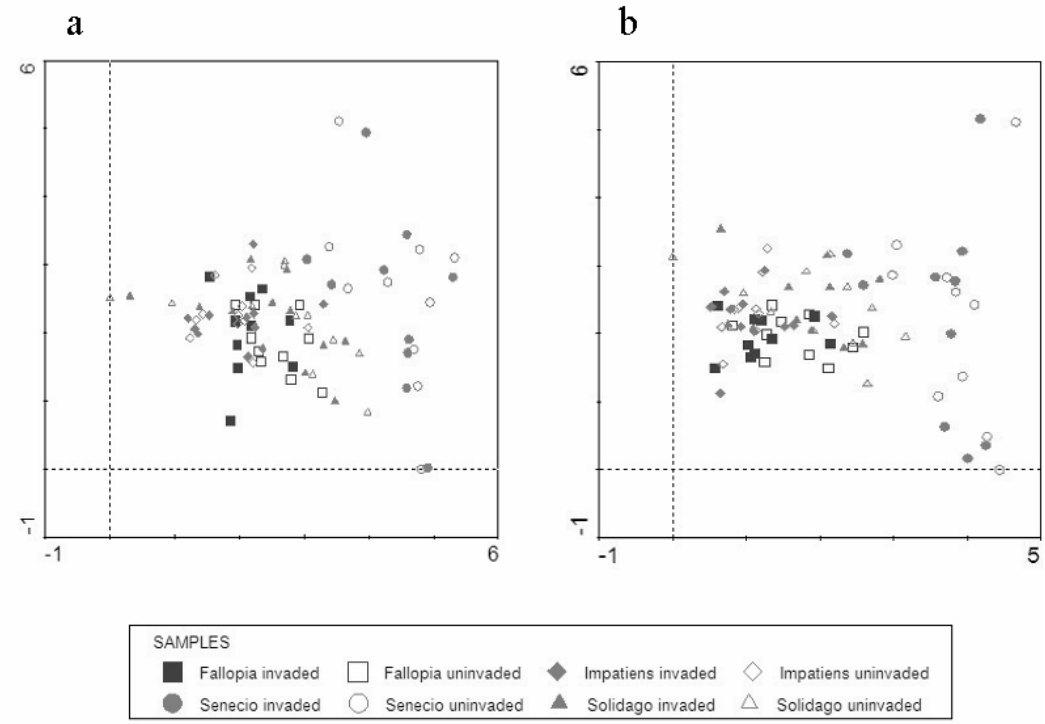


Figure 1 : Correspondence analysis ordination graph of plots realised from (**a**) species abundance data (**b**) presence-absence data.

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). These values compare to 110, 104, 97 and 122 species respectively in adjacent uninvaded vegetation. Mean species richness decreased significantly with invasion at the quadrat (1 m²) level for three species, *Fallopia* spp., *I. glandulifera*, *S. gigantea* but not for *S. inaequidens* (Table 2 and Table 3). While the mean species richness in uninvaded vegetation was similar for the four HIPS the impact of invasion varied considerably among the three species with significant effect : *Fallopia* spp. : 55% decrease; *S. gigantea* 33% decrease; *I. glandulifera* 16% decrease. Invasion also result in a significant decrease of species richness at the site scale (Table 2, statistics not shown). The same pattern was found with Shanon diversity indices (not shown).

Table 2: Mean species richness (+/-SD) per site and per m² for the four target HIPS.

		Mean species richness / site			Mean species richness / m²	
HIPS	**n**	**Invaded**	**Uninvaded**	**n**	**Invaded**	**Uninvaded**
Fallopia spp.	10	9.7 (5.1)	21.9 (10.9)	60	3.2 (2.6)	7.2 (3.7)
Senecio inaequidens	10	18 (7.3)	20.2 (9.2)	60	7 (3)	7.2 (3.4)
Impatiens glandulifera	11	14.2 (5.4)	19.9 (4.7)	66	5.8 (2.5)	6.9 (2.9)
Solidago gigantea	11	11.7 (4.6)	22.1 (9.05)	66	4.6 (2.3)	7.6 (3.5)

Table 3: p values for a two way ANOVA: effects of invasion, sites and their interaction on mean species richness.

HIPS	Invaded/Uninvaded	Site	Interaction
Fallopia spp.	<0.001	<0.001	<0.001
Senecio inaequidens	0.455	<0.001	0.005
Impatiens glandulifera	<0.001	<0.001	0.292
Solidago gigantea	<0.001	<0.001	<0.001

For the three HIPS that exhibited a global effect of invasion on species richness, a significant relationships between density (percentage cover) of the HIPS and the number of native species at 1m² scale was found for *Fallopia* spp. and *S. gigantea* but not for *I. glandulifera* (Fig. 2). 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.
In spite of the observed richness losses, a correspondence analysis using CANOCO 4.5 showed no clear differentiation between invaded and uninvaded sites for the four target HIPS (Fig. 1).

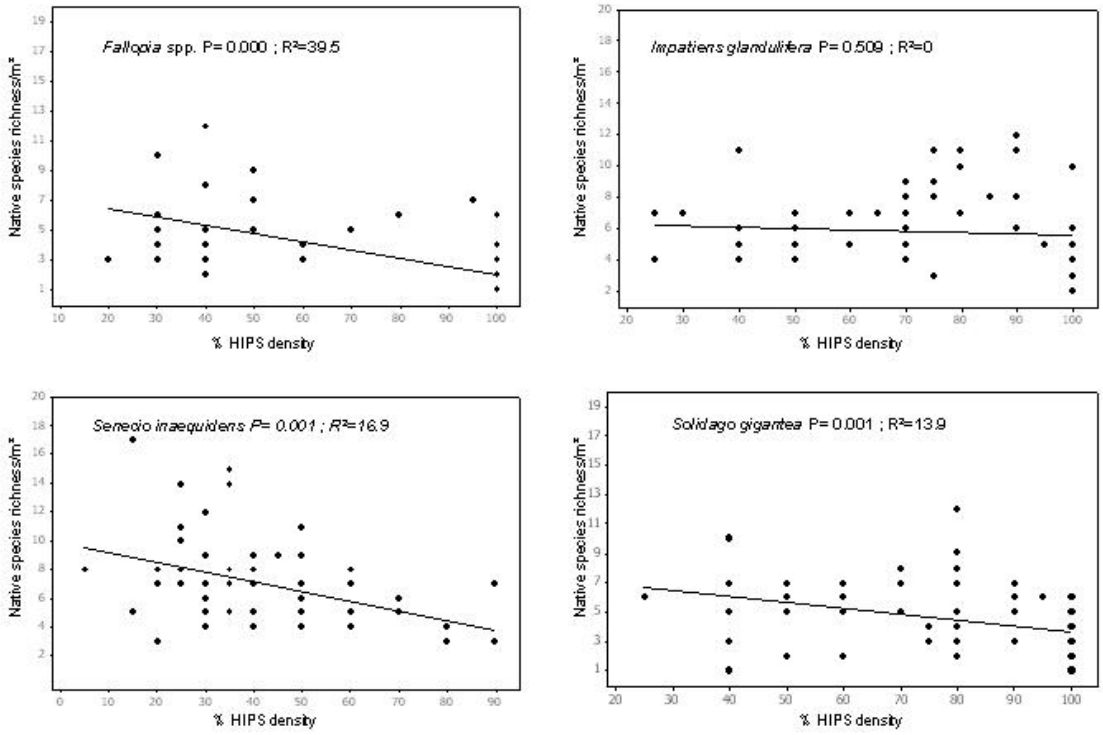


Figure 2: Linear regressions of native plant richness (per m²) vs. HIPS density (% cover) for the four target HIPS.

An indicator species analysis INDVAL (Dufrêne & Legendre, 1997) was performed to detect species significantly associated with the invaded/uninvaded sites. For the four HIPS, only the

HIPS themselves were considered indicator species in invaded vegetation. Therefore, no native species could be associated to the invaded plant communities. Moreover, only very few native species were considered as indicators in uninvaded vegetation and all of these were associated to the clonal species (*Fallopia* spp. n = 4, *I. glandulifera* n = 0, *S. inaequidens* n = 0 and *S. gigantea* n = 3). These results reflect the heterogeneity of invaded habitats.
Using the Jaccard index (JI), the ratio JI invaded/JI uninvaded was calculated to estimate the potential homogenizing effect of HIPS. A ratio >1 indicates homogenisation and <1 differentiation. Our results showed that HIPS induce variable effects and homogenisation was recorded in 44, 42, 53 and 64% of the occurrences (plots) respectively for *Fallopia* spp., *I. glandulifera*, *S. inaequidens* and *S. gigantea* (Fig. 3). In the case of *Fallopia* spp., *I. glandulifera*, *S. inaequidens*, invasion did not significantly increase the Jaccard index ($p > 0.05$), while it did so for *S. gigantea* ($p = 0.02$), meaning that the latter species has a significant homogenizing effect.
To consider habitats of high biological value a fifth HIPS was considered, *Cotoneaster horizontalis*. This species was found invading calcareous grasslands, habitats considered as biodiversity hotspots in temperate regions. The presence of *C. horizontalis* was shown to be associated with changes in both the structure and composition of the community by decreasing species richness and diversity, and affecting grassland specialist species (Piqueray *et al*., accepted).

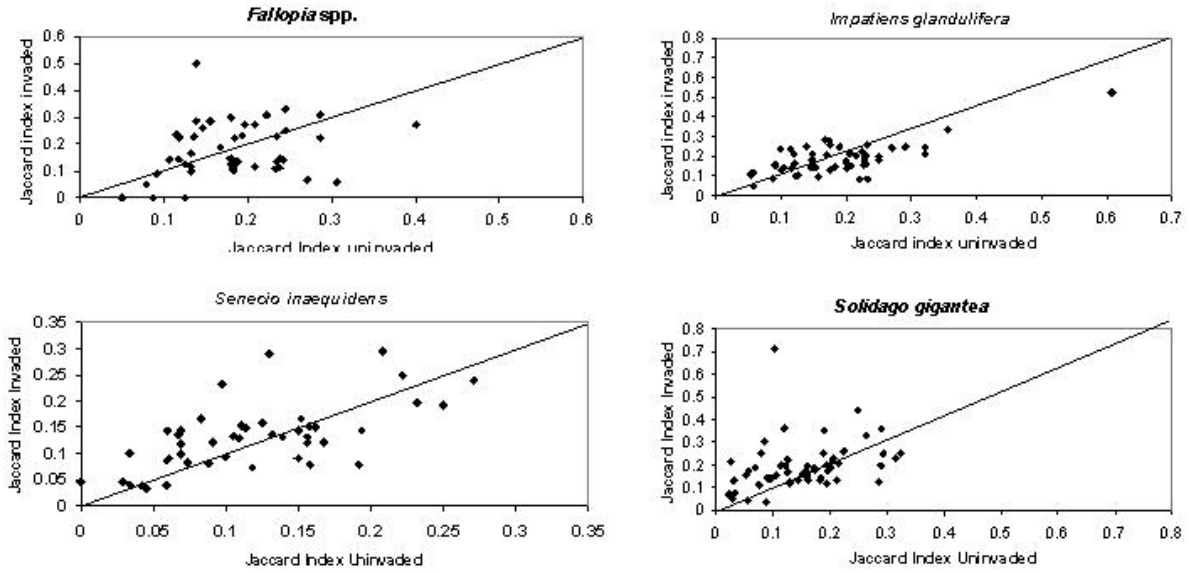


Figure 3: 20 out of 45, 23 out of 55, 24 out of 45 and 35 out of 55 comparisons between sites invaded respectively by *Fallopia* spp., *Impatiens glandulifera*, *Senecio inaequidens* and *Solidago gigantea*, and adjacent uninvaded vegetation in Belgium, occur above the line of isometry (slope = 1, y-intercept = 0), indicating that the invasion by these HIPS tends to increase similarity between plant communities in 42% to 64% of the occurrences.

***Conclusion**: In terrestrial communities, plant species richness loss was a common trend associated with invasion for* Fallopia *spp.,* I. glandulifera *and* S. gigantea*, but not for* S. inaequidens*. Impact increased with HIPS density except for* I. glandulifera*. Our results did not confirm the generally accepted hypothesis of plant communities homogenisation, except for* S. gigantea*.*

Task 1.2. Is species diversity affected by HIPS in aquatic communities? (VUB, month 5-12)

For this task, five target HIPS were chosen: *Ludwigia grandiflora*, *Myriophyllum aquaticum*, *Hydrocotyle ranunculoides*, *Egeria densa* and *Lagarosiphon major*.

A total of 35 water bodies were selected in Flanders and Walloon, mainly located in nature reserves where a relatively higher plant diversity could be expected. When available three plot types (2 x 2 m^2) were defined in each water body: plots in uninvaded water body (A), uninvaded plots (open water) in invaded water body (B), invaded plots in invaded water body (C). B and C plots are in the same water body, A plots are in a separate water body but in close vicinity (less than 200 meters).
In all plot types and each water body, the plant composition (emergent macrophytes, floating-leaved macrophytes and submerged macrophytes) and the percentage cover of native and alien species was monitored using a Braun-Blanquet method. Environmental variables (pH, dissolved oxygen, temperature, conductivity, depth, secchi depth) were measured *in situ* in each plot (three replicates).In each plot type, 10 subsamples of water, taken in different parts of the plot, were pooled into one sample for nutrient analysis: total phosphorus, soluble reactive phosphorus, NH_4^+, (NO_3 + NO_2^-) and chl a were determined in the lab using standard methods (APHA-AWWA-WEF, 1995).
Regarding the plant communities we observed that the presence of an invasive species was highly significantly and negatively correlated with both submerged (gamma r = - 0.64, $p < 0.001$) and emergent (gamma r = - 0.35, $p < 0.001$) vegetation cover, but that he submerged vegetation has significantly lower cover with increasing HIPS abundance (Fig. 4). *Ceratophyllum demersum* often is the most abundant species in type A plots but in C plots there is almost no submerged vegetation left.

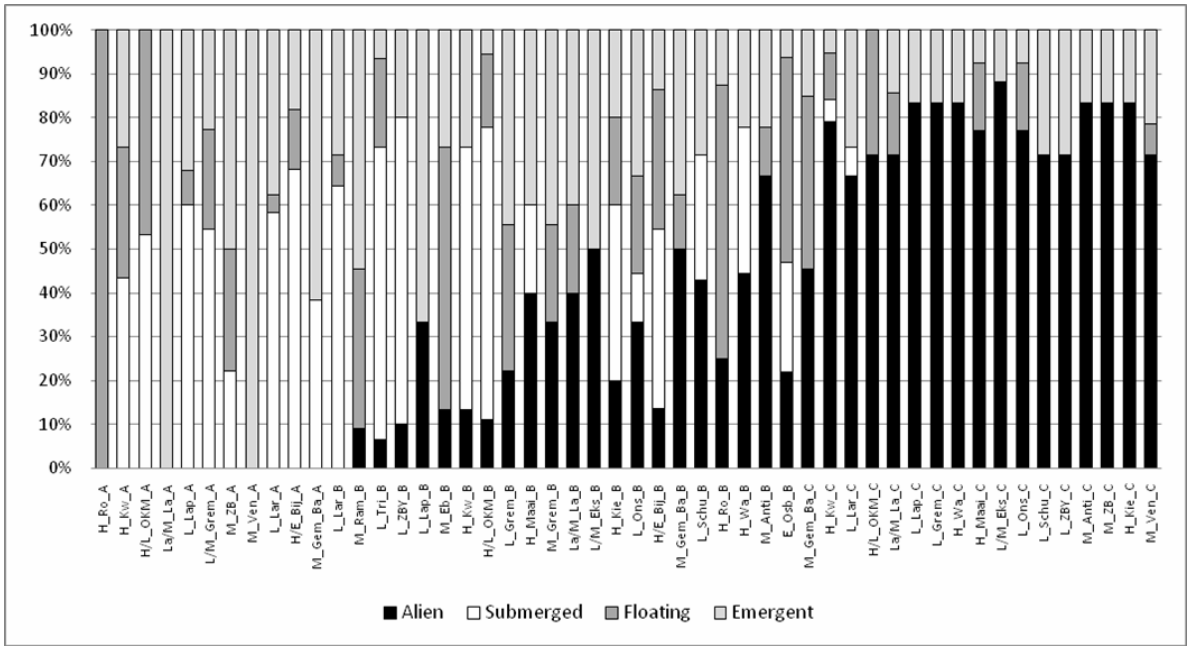


Figure 4: Relative machrophyte abundance in each site (native growth forms and aquatic HIPS)

A total of 12, 9, and 9 different species were recorded in C plots respectively for *L. grandiflora*, *M. aquaticum* and *H. ranunculoides*. These values compare to 24, 18 and 17 species respectively in B plots and to 20, 19 and 28 species in adjacent A plots. Mean species richness decreased significantly ($p < 0.01$) with invasion at the plot level for *L. grandiflora* and *H. ranunculoides* but not for *M. aquaticum* ($p = 0.08$) (ANOVA followed by a Tuckey post-hoc HSD test or Kruskal-Wallis test). (Table 4).

Table 4: Mean species diversity (range) and mean species richness (range) within each plot type per HIPS.

	Mean species richness			Shannon diversity index		
	A plots	**B plots**	**C plots**	**A plots**	**B plots**	**C plots**
H. ranunculoides (n = 38)	5.20 (2-13)	2.72 (0-4)	1.60 (1-3)	1.00 (0.54-2.18)	0.72 (0 -1.14)	0.32 (0-1.10)
L. grandiflora (n = 41)	6.78 (5-10)	4.10 (1-7)	2.23 (0-4)	1.24 (0.36-1.71)	1.02 (0-1.79)	0.67 (0-1.39)
M. aquaticum (n = 41)	4.00 (0-7)	2.78 (0-6)	2.00 (0-4)	0.83 (0-1.49)	0.79 (0-1.50)	0.40 (0-1.39)

Shannon diversity index was calculated per HIPS for each plot type (Table 4). One-way ANOVA and a Tuckey post-hoc HSD test (unequal n) revealed similar results as for species richness. There is significantly lower species diversity in C compared to A plots for *H. ranunculoides* and for *L. grandiflora* ($p<0.05$). Differences between A and C plots for *M. aquaticum* were marginally significant ($p = 0.06$).
Species richness per site showed on average a negative relationship to alien cover for each HIPS (Fig. 5).

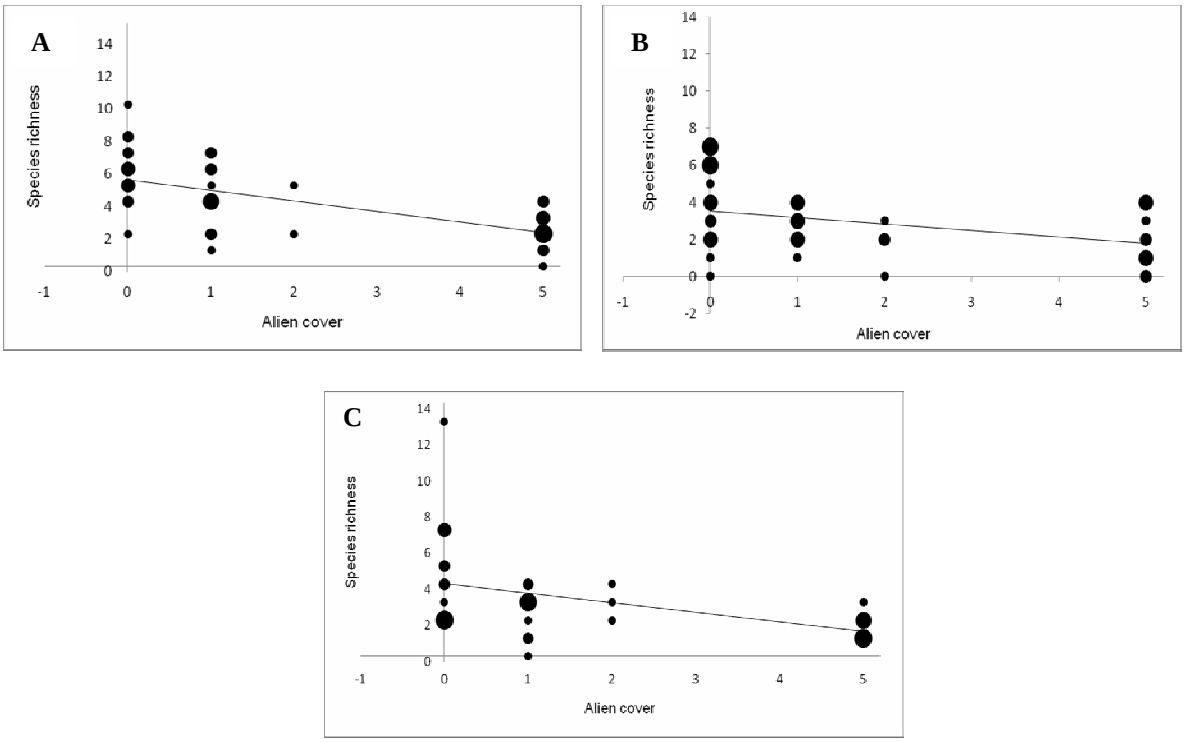


Figure 5: Species richness for the three target HIPS in function of alien cover. (**A**) *Ludwigia grandiflora* (gamma r = - 0.74, p < 0.001) (**B**) *Myriophyllum aquaticum* (gamma r = - 0.45, p < 0.01) (**C**) *Hydrocotyle ranunculoides* (gamma r = - 0.58 , p < 0.001). Dot size represents number of observations per site. X-axis (Braun Blanquet scale): 0 = no alien plants (A plots), 1 = 1-5% cover by HIPS, 2 = 5-25%, 3 = 25-50%, 4 = 50-75%, 5 = 75-100%.

A CCA (CANOCO 4.5, Fig. 6) showed a separation on the first axis (35.2% variation explained) between ponds invaded with *H. ranunculoides* and ponds invaded with *M. aquaticum* and *L. grandiflora*. *H. ranunculoides* seems to tolerate a wide range of nutrients with most of its ponds correlated with high nutrient levels (TP range: 10 µg/l – 1108 µg/l). The first axis thus represents a pollution gradient. It also represents a plant diversity gradient since the more diverse A ponds (see also Fig. 5) are on the left. The second axis (27.2% variation explained) gives a clear separation between univaded ponds (A plots) and highly invaded ponds (C plots) for all HIPS.

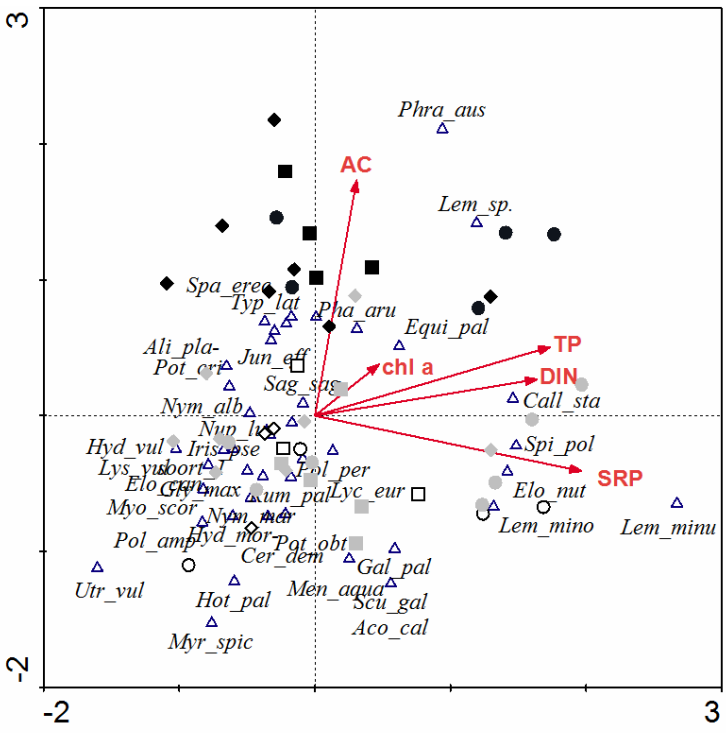


Figure 6: CCA ordination diagram with native species as species variables and TP, SRP, DIN, chl a (all log-transformed) and alien cover (AC) as environmental variables. ◊ = *Ludwigia grandiflora*, = *Myriophyllum aquaticum*, O = *Hydrocotyle ranunculoide;* white, grey and black signs represent A plots, B plots, and C plots, respectively.

Conclusion: *Aquatic HIPS have a significant negative impact on both native submerged (Elodeids) and emergent macrophytes, but the submerged vegetation has significantly lower cover with increasing HIPS abundance. Mean macrophytes species richness decreases with increasing HIPS cover. Multivariate analysis revealed a 'pollution-macrophyte diversity' gradient, with* H. ranunculoides *at higher eutrophication levels than* L. grandiflora *or* M. aquaticum.

WP 2 MECHANISMS OF HIPS IMPACT ON NATIVE PLANTS

Task 2.1 Elucidating direct niche impact via niche overlap (UA, month 27-42)

Task 2.2 Elucidating indirect impact mediated by pollinators

Task 2.2.a Elucidating indirect impact mediated by pollinators for terrestrial systems. (FUSAGx, month 13-42; UCL, month 13-42)

The aim of this task was to assess whether HIPS affect reproductive success of native plant species and whether those impacts are mediated through modification of pollinator services. The selected HIPS were *S. inaequidens*, *I. glandulifera*, *Fallopia* spp. and *Solidago* spp. The initial strategy was based on three steps: 1) selection of relevant native competitors (similarity of pollinator guild, ecological niche and phenological overlap), 2) field observations of pollinator interactions between natives and HIPS, and 3) controlled experiments to assess alteration of pollinator services. The work was greatly facilitated by preliminary activities of UCL on *S. inaequidens* and *I. glandulifera*.

For *S. inaequidens*, UCL found that the congeneric species *S. jacobaea* constitutes a relevant native competitor. The two species often occur in sympatry, have morphological floral similarities and have very similar visitor guilds (proportional similarity = 0.94; Vanparys *et al.*, 2008, UCL). Main visitors are Diptera, especially Syrphidae, and Hymenoptera. 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. Preliminary observations during summer 2007

and early summer 2008 confirmed the sharing of pollinator guilds between *E. angustifolium* and *I. glandulifera*: Hymenoptera (bumblebees and honeybees) represented 98% of their visitors.
This background allowed to apply the experimental approach in controlled garden already during year 2 of the project for *I. glandulifera* and *S. inaequidens* (subtask 2). 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 in year 2 as a first step to study species interactions in the field. The selection of native competitors for controlled experiments (phase 2, year 3 and 4) is performed by crosschecking pollinator guilds and flowering phenology between invasive and native species.

Subtask 1 : Field observation for *F. japonica* and *S. gigantea* – food web approach

In this ongoing task we address the following questions: 1) do native plants and HIPS share similar phenology? 2) what is the degree of pollinator sharing? 3) how do HIPS influence pollen transport efficiency?
Three study sites encompassing rich plant communities were selected per HIPS. 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 individuals were recorded for each observed plant-insect interaction: plants at the species level and category identification of the insect species (e.g. small Syrphidae). In addition, insects were collected on the same quadrats for further identification and for study of pollen transport. They were placed in tubes individually and directly killed in liquid nitrogen, in order to limit pollen losses. Flower abundance was estimated as percentage cover for each flowering species along the transect (Poaceae and non-flowering plants were excluded). Flower phenology was recorded for all species by random sampling of 10 plants per species on each site, and recording of the phenological stage of floral attributes (vegetative stage, flower bud, open flower, green fruit, mature fruit). On each site, observations were carried out every two weeks during the HIPS flowering period, adding up to three to four observations/site. Thousands of plant-insect interactions were recorded on each site and will be analyzed.
A total of 559 and 271 insects were collected in communities associated to *S. gigantea* and *Fallopia* spp. respectively. Those were pinned and are in the process of identification. Their bodies were rubbed with small agar cubes (Beatties technique) in order to gather the pollen they were carrying and those cubes are kept for further pollen identification.

Subtask 2: controlled experiments for *S. inaequidens* and *I. glandulifera*

In this ongoing task, we examine the impact of HIPS on reproductive success of native species and we provide a detailed analysis of the process that conduct to those impacts. 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 – S. jacobaea*. 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 (Fig. 7). All plants were grown in pots.

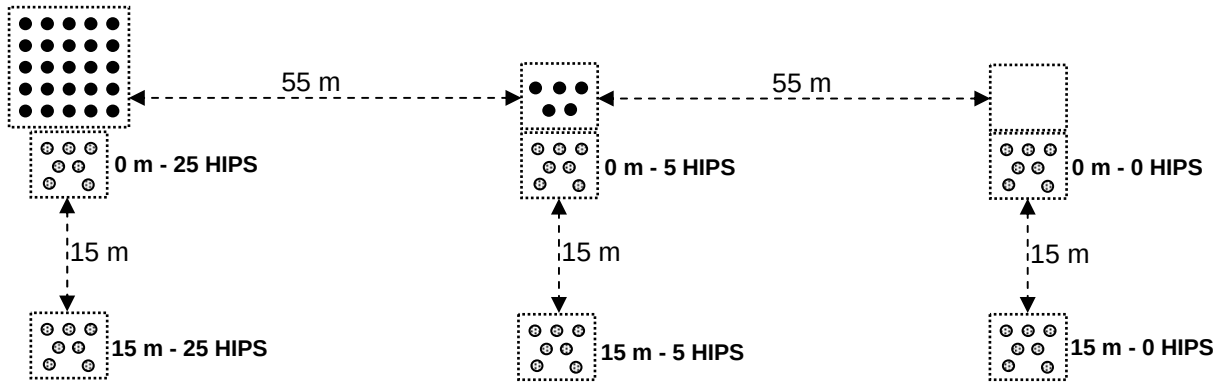


Figure 7: Design for the controlled experiment in experimental parcels. Each dot represents one plant of HIPS (black dots) or native species (grey dots).

Each experiment was constituted of two concomitant replicates (sites) situated around Louvain-la-Neuve at a distance of 2.6 km of each other. Both sites were mown grassland of approximately 120 m length and 55 m width. They were exposed to the sun all day long (no shading from trees). Site A was situated between a golf course and meadows. Site B was situated between a cereal field and a corn field. In each clump of plants, insect observations during 10 min-period (number of visits, visitor identity, time spent per flower or capitulum, and number of visited flowers or capitula per trip) were performed in 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). In order to measure the reproductive success of the native species by the seed set (number of seeds / number of ovules), flower buds or capitula without opened florets were marked on each native plant the first day of the experiments. For *E. angustifolium* and *A. napellus*, visitors were caught and flowers at the female stage (protandrous plants) were sampled. The pollen transfer quality will be estimated by discrimination of the native and the alien pollen grains deposited on insect bodies and on flower stigmas.

a) *Impatiens glandulifera*

Fragments of rhizomes of the two native species (*E. angustifolium* and *A. napellus*) were collected in three populations between April and May 2008, and *I. glandulifera* was cultivated from seeds. The experiment with species pair *I. glandulifera – E. angustifolium* took place from 22 to 29 July 2008. The experiment with the second pair (*I. glandulifera – A. napellus*) was started directly afterwards until August 11th 2008. A total of 288 10 min-periods of insect observations per native-invasive pair were performed and 2606 insects were individually censured on all three plants species. The seed set of *A. napellus* was estimated on 127-242 ripe fruits per clump collected in September.

I. glandulifera, *E. angustifolium* and *A. napellus* shared the same pollinator species but the relative frequencies of insect categories varied. The main visitor categories were honeybee, bumblebee (mainly *Bombus pascuorum* and *B.* type *terrestris*), and to a lesser extend Diptera (syrphids and flies; 1-15% of the visitors). Between the two sites, no differences were observed in the visitor guilds for species pair *I. glandulifera – E. angustifolium* (three insects categories: honeybee, bumblebee and other insects; χ^2=3.55, p=0.17; proportional similarity of the visitor guild (PS)=0.88) whereas slight differences were observed for species pair *I. glandulifera – A. napellus* (χ^2=139.1, p<0.001; PS=0.74) because honeybees were proportionally more abundant at site B (66 *versus* 41%). In control clumps (0 HIPS at 0 and 15 m), *E. angustifolium* was mainly visited by honeybees (site A: 76%, B: 84%) similar to *A. napellus* (site A: 60%, B: 87%), whereas *I. glandulifera* was mainly visited by bumblebees (80-95%). The visitation rate (number of visitors per 10 min-period / number of opened flowers) was significantly higher for the alien species (5.9 time higher than *E. angustifolium*, χ^2=80.7, p<0.001; 4.3 time higher than *A. napellus*, χ^2=74.5, p<0.001). The high attractiveness for insects of *I. glandulifera* is often explained by its greater nectar production compared to native plants (Chittka & Schürkens,

2001). In this study, we chose native plants with a high nectar production, particularly *A. napellus*, which had in controlled growth chamber a daily sugar production per flower of 1.9 mg against 2.2 mg for *I. glandulifera*. All nectar types had on average the same total sugar percentage (48-52%) but their sugar composition was very different. Nectar of *I. glandulifera* was mainly composed of sucrose (96%) whereas those of native species were mainly composed of fructose (*A. napellus*: 57%; *E. angustifolium*: 65%).
The presence of *I. glandulifera* had no clear impact on the number of visitors on *A. napellus* whereas a positive impact (facilitative effect) on *E. angustifolium* was observed at short distance (χ^2=13.4, p=0.0012). At 0 m, the number of visitors on *E. angustifolium* increased with an increasing number of HIPS (0 – 5 – 25 HIPS: 8.2 – 10.7 – 12.5 visitors; χ^2=15.5, p=0.0004). This effect of the alien species on the pollination of the native species was not found at the 15 m distance (χ^2=4.5, p=0.10). Surprisingly, the different insect categories did not react similarly. The number of honeybee visits, the main pollinators of *E. angustifolum*, highly decreased at the highest density of HIPS (0 – 5 – 25 HIPS: 6.6 – 6.2 – 3.7 visitors; χ^2=11.3, p=0.0035), whereas the number of bumblebee visits, the main pollinators of *I. glandulifera*, increased proportionally with the number of HIPS (0 – 5 – 25 HIPS: 0.6 – 3.6 – 7.8 visitors; χ^2=60.3, p<0.001). For both native species, the presence of *I. glandulifera* had an influence on the relative frequencies of their visitor categories (e.g. site A, *E. angustifolium*: χ^2=250.1, p<0.0001; *A. napellus*: χ^2=65.9, p<0.0001) as the bumblebee proportion increased against the honeybee proportion. Therefore the proportional similarity of the visitor guild (PS) between the native and the alien species increased. This impact was more important at 0 m (*e.g.* site A, *E. angustifolium*, 0 – 5 – 25 HIPS: PS=0.10 – 0.30 – 0.66; χ^2=147.1, p<0.0001) than at 15 m (0 – 5 – 25 HIPS: PS= 0.14 – 0.17 – 0.24; χ^2=8.7, p=0.068).
Insect foraging behaviour *on* both native species in terms of time spent per flower was not influenced by the alien presence. Concerning the number of native flowers visited per trip by the different insect categories, HIPS presence did not influence the foraging behaviour of honeybees on both native species but bumblebees visited less flowers of *E. angustifolium* (in order to increase the number of data with 0 HIPS, data from clumps with 0 HIPS were pooled with data from clumps with x HIPS at 15 m; 0 – 5 – 25 HIPS: 19.7 – 7.8 – 8.3 flowers; χ^2=12.3, p=0.0021). Actually, at 0 m we often observed bumblebees from *I. glandulifera* which visited only 1-3 flowers of *E. angustifolum* before returning on the alien plant as if they visited the native plant by mistake. In the experiment with *A. napellus*, no changes in the foraging behavior of bumblebees were observed (χ^2=2.01, p=0.85) but the main *Bombus* species was not the same in the two experiments. On *E. angustifolium* 80% of the bumblebees were *B. terrestris* whereas on *A. napellus* 87% were *B. pascuorum* and this last species is well known to be faithful (Le Cadre, 2005).
For *A. napellus*, a particular insect behavior was observed. A large proportion of honeybees (84%) and some bumblebees (33%) stole the nectar of at least one flower per trip. They slipped under the upper sepal to drink nectar without touching male and female organs. This behavior was not known for this species. Usually, nectar robbers make a hole in the upper sepal (Le Cadre, 2005). In this study, no hole was observed. Despite the robbery of nectar and a possible deposit of alien pollen on *A. napellus* because of switches of bumblebees between alien and native species, the seed set of *A. napellus* was high (≥ 70%) and not influenced by the HIPS presence (F=1.01, p=0.42).

b) *Senecio inaequidens*

For *S. inaequidens*, one- or two-years-old plants with flowers buds were selected in four populations. Plants of *S. jacobaea* were collected at the rosette stage to avoid root stress during the flowering period. Approximately 300 rosettes were selected in five populations in June, but only 18 individuals flowered in August. Therefore the experimental design was limited to one distance: 0 m between native and invasive species and the 18 individuals were equally arranged in the six clumps (three per site). To complete the clumps (seven plants), fresh flowering stems from individuals growing along roadsides of Louvain-la-Neuve were cut and placed in water vases. A total of 180 10 min-periods of insect observations were performed between 11 and 28 August and 550 insects were individually censured on both

plants species. The seed set of *S. jacobaea* was estimated on 8-17 ripe capitula per clump collected in early September.
No differences in the visitor guild were observed between both *Senecio* species. The main visitor categories were large-size syrphids (63%) and to a lesser extent small-size syrphids (22%), and other Diptera (12%). Some Hymenoptera and Lepidoptera (3%) were also observed. The proportional similarity (PS) of the visitor guild between the two plant species was very high, and reached 0.95 in each site. Between the two sites, slight differences were observed in the visitors guilds (χ^2=54.4, p<0.001; PS for *S. jacobeae* = 0.83) because Diptera, other than syrphids, were proportionally more abundant at site B while small-size syrphids were less frequent. The visitation rate was significantly higher for *S. inaequidens* (0.16 *versus* 0.7; T=-5.4, p<0.001). Until now, we did not find an explanation for this. *S. inaequidens* did not exhibit a higher nectar production or a different sugar composition, only a slightly higher sugar concentration (76 *versus* 69%), and no role of the floral display was detected (Mahaux, 2008, UCL).
The presence of the invasive plant had no impact on the number of visitors per 10 min-period on *S. jacobaea* (F=0.22; p=0.80). At site A, an effect of the number of *S. inaequidens* was found on the relative frequencies of the three main visitor categories of *S. jacobeae* (χ^2=12.6, p=0.01; the proportion of large-size syrphids increased with the number of HIPS), but no difference was detected at site B (χ^2=3.7, p=0.44). Insect foraging behaviour *on S. jacobaea* was not influenced by the number of *S. inaequidens*, in terms of time per capitulum (all insect categories pooled, χ^2=0.47, p=0.79) but was influenced in terms of number of visited capitula per trip (all insect categories pooled, χ^2=8.0, p=0.018). The number of native capitula visited per trip by an insect decreased with the number of HIPS ((0 – 5 – 25 HIPS: 4.2 – 3.6 – 3.1 capitula). Despite a probable inter-specific pollen transfer between the two *Senecio* species (visitors switching between the two species were often observed), the seed set of *S. jacobaea* was not significantly influenced by the number of HIPS (F=2.39, p=0.10).

***Conclusion**: For two HIPS*, I. glandulifera *and S. inaequidens, we identified three native counterparts that share similar habitats and insect visitors and have overlapping flowering periods:* E. angustifolium*,* A. napellus *and* S. jacobaea*. We showed that pollinator-mediated impacts of HIPS on native species were specific to each pair of native – invasive species. On the other hand, impacts detected with our experimental design were often proportional to the number of HIPS and decreased with the distance between native and invasive species . The first results indicate that the observed negative impacts of the HIPS on native cover may not be realized via this indirect pathway.*

Task 2.2. b Elucidating indirect impact mediated by pollinators for aquatic systems (VUB, month 31-35)

Task 2.3 Elucidating indirect impact via soil modification in terrestrial systems.

Task 2.3.a Elucidating indirect impact via soil modification in terrestrial systems. Understanding mechanisms of soil modification. (ULB, month 1-21)

A previous project (INPLANBEL) indicated that soil modification can relate to both N and P, but that the element involved may vary with the HIPS concerned. This was the basis for focusing within Alien Impact on:

(1) Organic matter and nitrogen cycling in *F. japonica*.

This study site is located in the nature preserve "Les Marionvilles" in Saint-Ghislain (Hainaut, Belgium). There, *F. japonica* invades a rough grassland which was used as control. We studied the decomposition of (1) senescent leaves of *F. japonica*, (2) senescent stems of *F. japonica* and (3) a mixture of leaves of two dominant species of the resident vegetation (*Eupatorium cannabinum* and *Calamagrostis epigejos*). The three litter types were put into nylon bags and placed in each vegetation type (*F. japonica* and resident vegetation). Every two months during one year, one litterbag of each type was sampled in each plot. The remaining

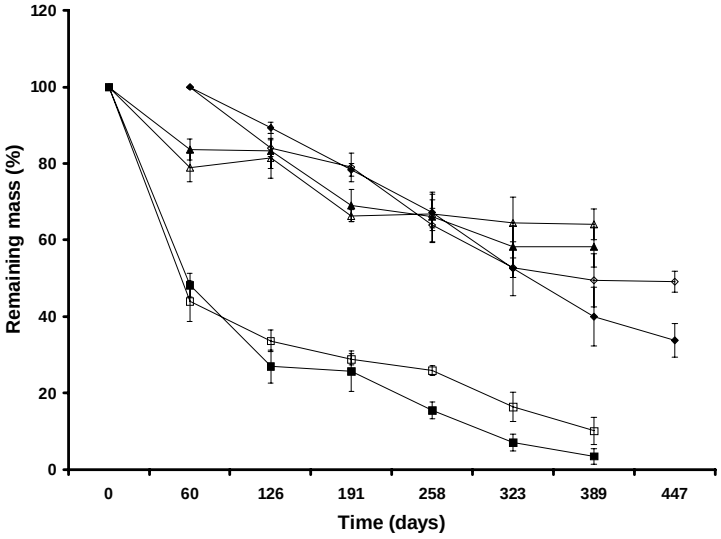
material was cleaned of soil particles, oven-dried and weighed. C and N content of the remaining litter was assessed using a CN analyzer (TruSpec analyzer CN Leco, USA). The initial content in lignin and cellulose was also analysed. Decomposition kinetics of each litter type in each environment was analysed and k coefficients of decomposition were calculated.
In order to assess N pools and fluxes in the plant-soil system in invaded and uninvaded plots, soil and plant samples (living leaves, living stems, stem litter, leaf litter and standing dead stems for *F. japonica* and total aboveground biomass for uninvaded plots) were collected in March, June, August and November. Plant samples were oven-dried and weighed. Plant and soil samples were analyzed for N and C concentration.
The different types of litter tested had a contrasting chemical composition, with a very high C/N ratio for both *F. japonica* litter types (leaves and stems) compared to the native mixture. The C/N ratio of *F. japonica* stems was two-fold higher compared to leaves. Also the lignin concentration in *Fallopia* organs was higher compared to the native mixture (Table 5). The cellulose concentration in *F. japonica* stems was higher compared to both *F. japonica* leaves and the native mixture.
There was a significant variation in decomposition rate among litter types (significant litter x time interaction, Fig. 8) with native species mixture decomposing faster (four times) than both leaves and stems of *F. japonica*. There was a significant litter x invasion interaction due to slightly higher decomposition rate in invaded plots for *F. japonica* stems and for the native litter.

Table 5: Chemical composition of the three litter types.

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

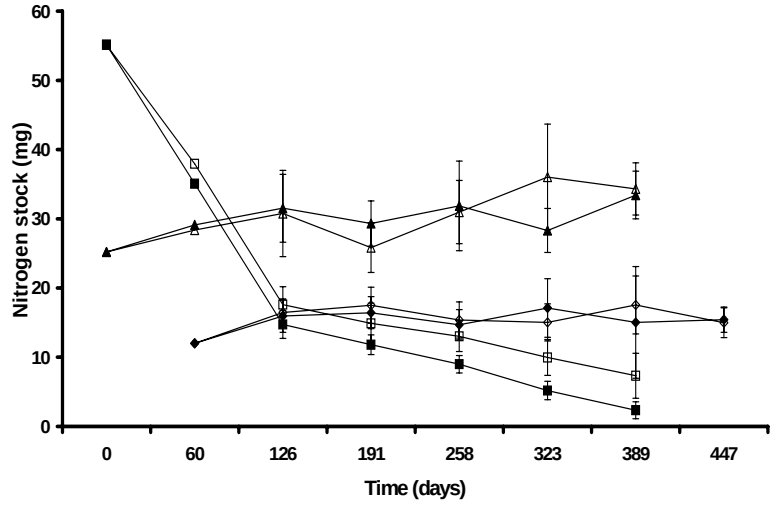
Mean ± standard deviation (n = 2).

Nitrogen stock in litterbags decreased throughout the experiment for the native litter indicating net mineralisation (Fig. 9). In contrast, for *F. japonica*, N stock did not vary much, indicating N immobilisation.
N fluxes in *F. japonica* plots are summarized in Fig. 10. A striking result is the huge amount of N allocated to shoots (79 g m^{-2}) with 56 g m^{-2} allocated to leaves and 23 g m^{-2} to stems and branches. Then leaves and stems translocated respectively 47 and 14 g m^{-2} back to rhizomes resulting in 79% of the aboveground N retranslocated. Total aboveground N stock was always much higher in *F. japonica* plots than in uninvaded plots. The protocol of nitrification measurements did not work so no data are yet available.



	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 8: 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 showes the three way ANOVA results for decomposition of the three litter types during one year. Degree of freedom (df), Mean square (MS)
and significance level: *: p<0.05, **: p<0.01, ***: p<0.001.



	df	MS stock N (mg)
Litter	2	3,00 ***
Invasion	1	0,16 ***
Time	4	0,17 ***
Litter*Invasion	2	0,20 ***
Litter*Time	8	0,21 ***
Invasion*Time	4	0,04 ***
Litter*Invasion*Time	8	0,03 ***

Figure 9: Evolution of the N stock (= remaining mass x N concentration) in *F. japonica* leaves (triangles), stems (diamonds) and indigenous litter (squares) during decomposition in invaded (black) and uninvaded (white) environment. Values are means ± standard deviation.
The table showes the three way ANOVA results for nitrogen dynamics of the three litter types during decomposition. Degree of freedom (df), Mean square (MS)
and significance level: *: p<0.05, **: p<0.01, ***: p<0.001.

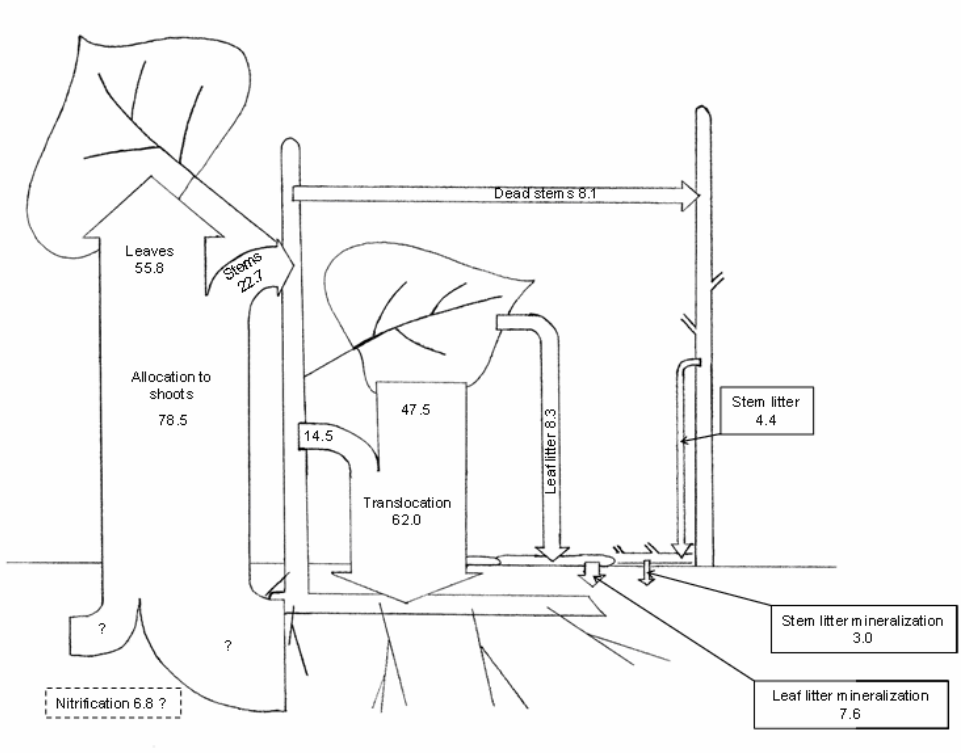


Figure 10: Estimated annual N-fluxes (g m^{-2}) in *Fallopia* stands.

2) Impacts of *S. gigantea* on phosphorus : seasonal effect on soil phosphorus pools and fluxes.

The study site is located in Kraainem (Vlaams Brabant, Belgium). There, monospecific stands of *S. gigantea* invade a native mesic grassland. 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 resident vegetation. Different extraction methods were used to assess specific fractions of phosphorus of contrasting bioavailability. To that end, soil cores were collected on five dates (July, September 2006, January, March and May 2007) in five plots for each type of vegetation, with six soil cores per plot. P pools in standing biomass including belowground organs were also assessed. Aboveground vegetation samples (living leaves, living stems and litter) were collected at three constrasted phenological states (May, August, and November 2007) and root samples were excavated on two dates (August and November 2007). Soil P pools and fluxes were calculated.
There were systematic differences in P availability and pH between the topsoil of invaded and uninvaded plots. Soil pH was lower in invaded plots at all dates (Fig. 11), while labile phosphorus pools (Resin-Pi, Bicarb-Pi and NaOH-Pi) were 20-30% higher throughout the season (Fig. 12). We also found a significant decrease (30%) of alkaline PME in invaded stands. In summer, *S. gigantea* stands had much higher standing biomass compared to the control plots. The stocks of P in belowground organs of *S. gigantea* showed a more than two-fold increase in autumn, which was not observed in the control (Fig. 13).

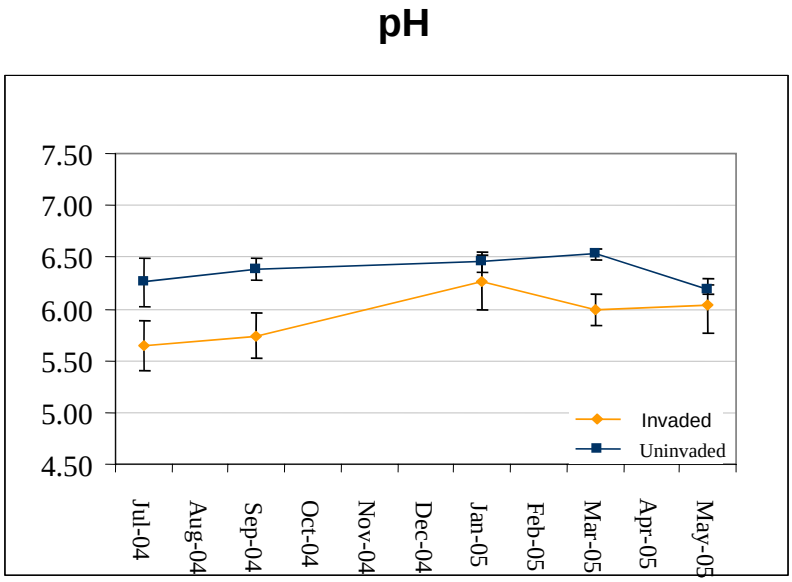


Figure 11: Seasonal variation of soil pH in plots invaded by *Solidago gigantea* and adjacent, uninvaded plots. Means (n = 6) and standard deviations.

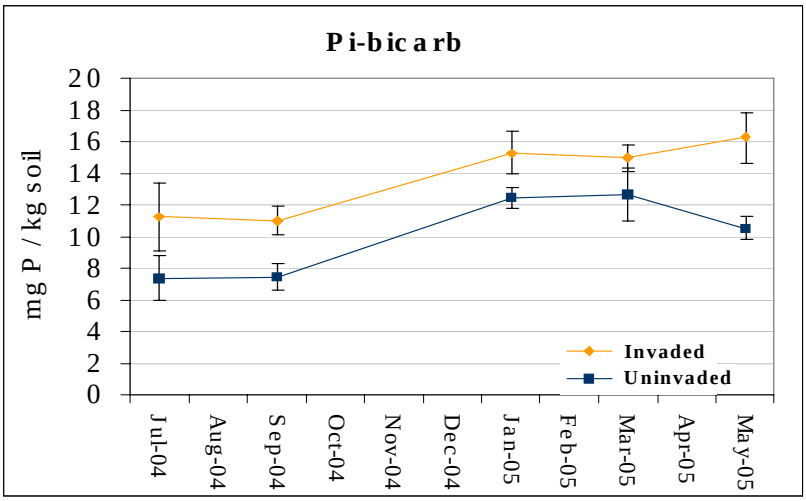


Figure 12: Seasonal variation of soil inorganic P extracted by bicarbonate (0-10 cm) in plots invaded by *Solidago gigantea* and adjacent,uninvaded plots. Means (n = 6) and standard deviations.

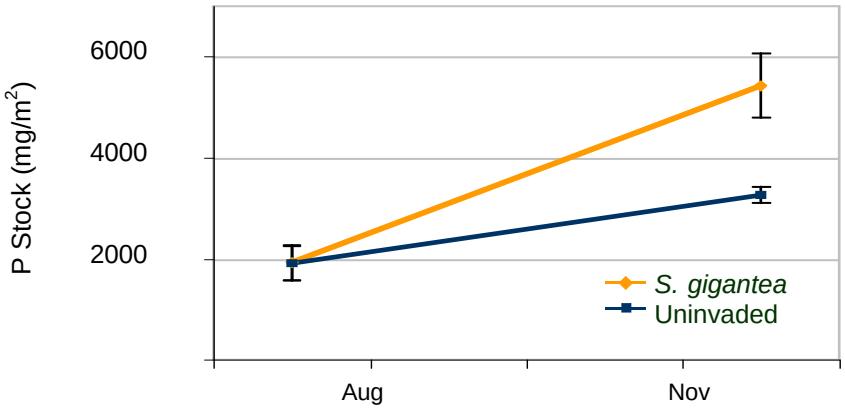


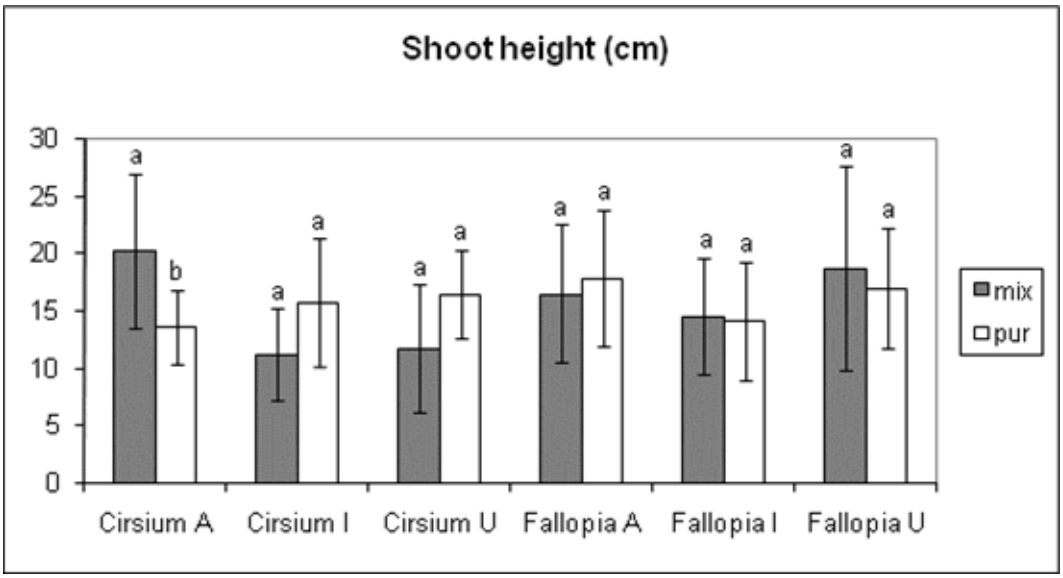
Figure 13: P stock in belowground organs (g m^{-2}). Stock were calculated as P concentration x mass. Means (n = 6) and standard deviations.

Conclusion: F. japonica *produces recalcitrant litter that decomposes slowly compared to the native mixture. The species also seems to influence environmental conditions for decomposition. Concerning N dynamics, immobilization occurs in* F. japonica *litter, while the species has an efficient resorption in belowground organs.* S. gigantea *increases the labile phosphorous pool in invaded plots, most likely due to a pH decrease, its fine root dynamics and a phosphatase decrease.*

Task 2.3.b Impacts of soil modification on competition between native and alien species. (ULB, month 7-30)

Previous work (Inplanbel project) suggested that invasive species might compete through soil modification. A competition experiment between *F. japonica* and the native species *Cirsium arvense* is currently being 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, soil from invaded plots amended with charcoal). The topsoil necessary to conduct the experiment has been removed from the 0-10 cm layer in the same *F. japonica* site as in task 2.3.a. Two pure cultures and a 50:50 mixture are used with 10 replicates (pots) per treatments (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). A non-destructive growth measurement was performed in June 2008: number of leaves, axes length (main stem + ramifications), length and width of the largest leaf. Further non-destructive measurements will be made at three-month intervals. The plants will be harvested after two years and weighed. From the first measurement period, the average of above mentioned parameters was computed for each species in each pot. For each of the two species separately, a two way ANOVA was performed on each parameter with soil (uninvaded, invaded and invaded + charcoal) and type of culture (pure or mixed) as fixed factors. The ANOVA was followed by a Newmann-Keuls post-hoc test to detect significant differences between treatments.
For axes length, the two way ANOVAs showed no significant soil or culture type effect. However, for *C. arvense* only, a significant soil x culture type interaction ($F = 8.02$, $p < 0.001$) was found. This significant interaction reflects the different behaviour of *C. arvense* in pure vs. mixed culture between the different soils (Fig. 14).

A



B

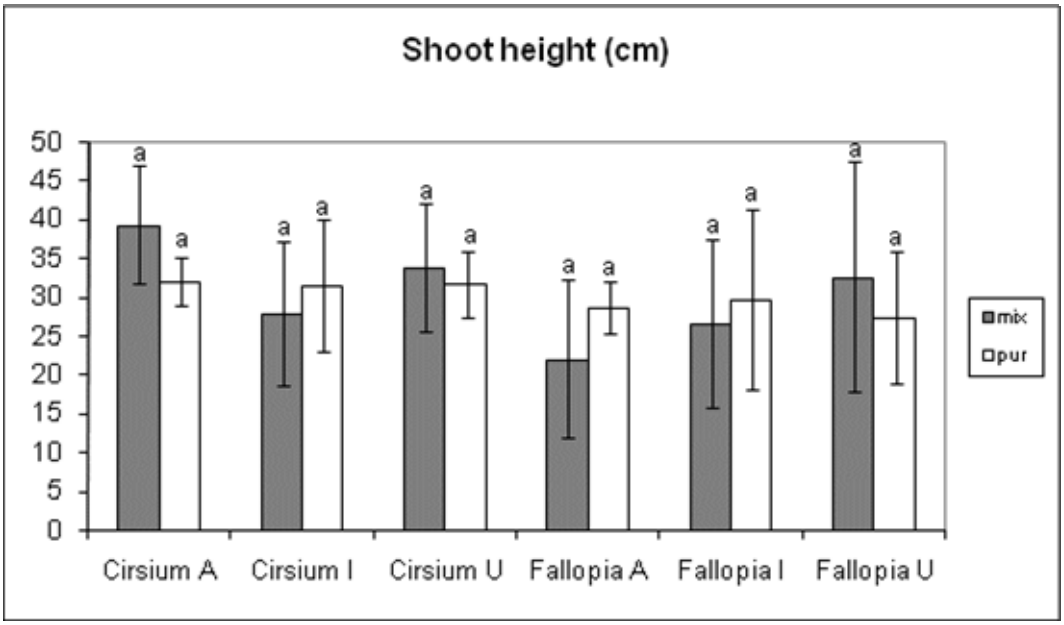


Figure 14: Mean shoot height (cm) of *Cirsium arvense* and *Fallopia japonica* in function of substrate type in pure (white) and mixed culture (grey). (**A**): Plants were cultivated on soil from uninvaded (U) plots and invaded (I) plots, amended or not with charcoal (A). Means with different letters are significantly different at the $p \leq 0.05$ level as determined by the student - Newman - Kuels (SNK) test. Whiskers are standard deviations (n = 10). A: Non destructive data from june 2008. (**B**): Non destructive data from october 2008.

Both in invaded and uninvaded soils, axes length tended to be higher in pure culture compared to mixed culture while the opposite pattern occurred in invaded soil amended with charcoal. The difference between pure and mixed culture was only significant in soil with charcoal (Newman-Keuls test, $p = 0.028$). The trend observed for *F. japonica* was exactly the opposite of that observed for *C. arvense* (higher axes length in mixed culture in invaded and uninvaded soil but lower axes length in mixed culture in soil amended with charcoal). However, for *F. japonica*, no significant effect was found. The pattern observed with axes length was also found for number of leaves and for the product of length and width of the largest leaf (not shown). For the second non - destructive measurements (October 2008), only data of *C. arvense* on axes length showed a significant soil x culture type interaction ($F = 5.20$, $p < 0.01$). Indeed, in Invaded soil, axes length tended to be higher in pure culture compared to mixed culture while the opposite pattern occurred in invaded soil amended with charcoal.

***Conclusion**: No significant difference was observed between plant performance in invaded and univaded soils. But C. arvense decreased competitive superiority of the invasive species in presence of charcoal. This points to allelopathic effects.*

WP 3 IMPACTS AT OTHER TROPHIC LEVELS

This section explores possible proliferation of impacts through the foodweb.

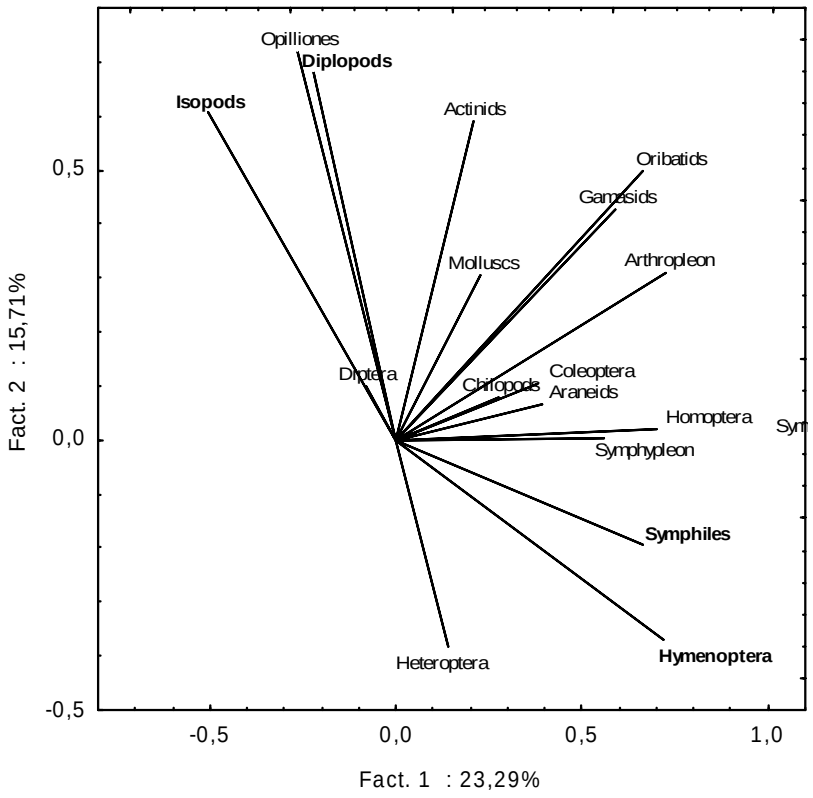
Task 3.1. Terrestrial: Impacts of HIPS on soil fauna. (ULB, month 13-36)

Impact of *F. japonica* and *S. gigantea* on soil fauna.

The study site for *F. japonica* was the same as for the litterbag experiment (task 2.3.a). Two sorts of fauna were collected, i.e. the endogeic fauna and the epigeic fauna. For the endogeic fauna, soil cores (diameter: 8 cm, depth: 0-4 cm and 4-8 cm) were taken every three months for one year with five replicates in each plot (invaded and uninvaded). Soil

microarthropods were extracted from the fresh soil cores by the Berlèse-Tullgren method. For the surface active macrofauna, pitfall traps were used (five traps in each type of vegetation). Samples were collected every three weeks for one year. All the invertebrates collected were identified at the family level and the data are presented as counts of individuals for each taxonomic group. In addition, earthworm extractions were performed in October 2007 and April 2008 using formaldehyde (0.04%). Fourteen quadrats (30 x 30 cm) were sampled from *F. japonica* and native vegetation, respectively. Earthworms were identified at the species level, counted and weighted. The soil fauna extraction protocol has been replicated in two other sites (Jemeppe –sur- Sambre and tervuren)(2008).
To assess the soil fauna under *S. gigantea*, four sites were selected: Kraainem as in task 2.3.a, and two new sites (Boisfort and Marche - les - Dames). In those, ten soil cores (diameter: 20 cm, depth: 0-5 cm) were collected and five pitfall traps were placed in each plot (invaded and uninvaded). Sampling was repeated at two dates, i.e., April and October 2008. Data analysis still in progress.
For all dates, the total number of individuals found in the soil of invaded plots was dramatically lower (a decrease until 60%) compared to uninvaded plots. However, no significant difference in faunal diversity was found between both types of vegetation. Still, some groups were completely absent from invaded plots, including Hymenoptera (ants) and Homoptera (Aphids). In contrast, higher numbers of woodlice (Isopoda), millipedes (Diplopoda), Opiliones (Aranaeids) and oribats (Acarida) were collected in invaded plots. Other groups (Gamasida (Acarida), Actinedida (Acarida), Chilopoda and Collembola (Springtails)) were not different between invaded and uninvaded plots. Multivariate analysis confirms distinct faunistic assemblages in invaded and uninvaded plots (Fig. 15). For example, isopods or diplopods that have affinity for shaded and humid environments (the invaded plots) contrast with thermophilous organisms such as the ant *Lasius flavus* and the associated aphids which were totally absent from invaded plots. Another interesting result concerns differences in earthworms species, with species associated with moist environment (i.e. *Lumbricus terrestris* and *Dendrobaena subribicunda*) only being present in the invaded plots in contrast with grassland species (*Lumbricus castaneus*) which were only present in the resident vegetation.

A



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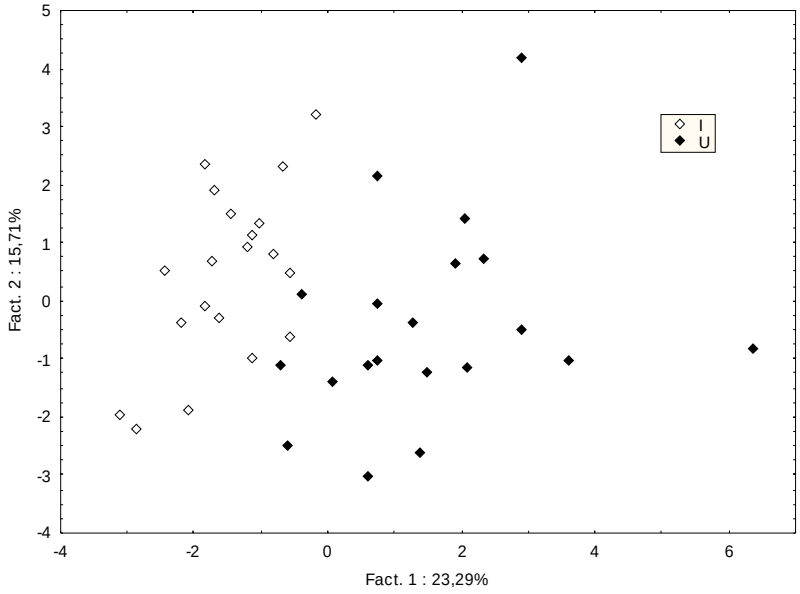


Figure 15: Principal Component Analysis (PCA). (**A**) Projection of variables (taxonomic groups) on PC1 and PC2 for the global fauna (epigeic and endogeic), all four dates pooled.
(**B**) Projection of invaded (white) and uninvaded (black) plots.

Conclusion*: Soil fauna density strongly declined under the* F. japonica *canopy. Although soil fauna diversity was not affected, distinct faunistic assemblages (including earthworms) were found in invaded and uninvaded plots. The data point to microclimatic differences being responsible.*

Task 3.2. Aquatic: Impact of HIPS on other trophic levels in water/sediment. (VUB, month 19-24, 36-38)

In 22 ponds the sediment (detritus) macro-invertebrates were sampled during April 2008 on 14 different sites. Ponds were separated in non-invaded ponds (A plots from Task 1.2) and invaded ponds (either *M. aquaticum*, *L. grandiflora* or *H. ranunculoides* was present, B/C plots from Task 1.2). During spring the vegetation is not fully developed, which enables efficient sediment sampling for macro-invertebrate diversity and abundance.
The benthic macro-invertebrates were sampled with a corer (Ø= 55 mm) at 10 cm depth and a volume of 237.5 cm³ sediment per sample was taken. Ten samples were taken among a transect for each pond and each sample was fixed in the field with 10% formol solution. Environmental variables of the water column were measured *in situ* (conductivity, pH, dissolved oxygen, temperature, secchi depth, redox potential). Afterwards each sample was rinsed and passed through different mesh sizes (2 mm, 1 mm, 500 µm, 350 µm) in order to separate the macro- invertebrates from the sediment. Macro-invertebrates were preserved in 70% alcohol and identified at family level. Identification of crustaceans, some diptera and some oligochaeta, was done at genus level.
Macro-invertebrate data were analysed per HIPS and the non-invaded ponds were considered as one group. The most abundant groups in both invaded and uninvaded ponds were Tubificidae (29.25%), Chironomidae (20.81%) and Naididae (17.73%). The invaded ponds seem to have a lower macro-invertebrate abundance (Fig. 16 A) and mean numer of taxa (Fig. 16 B) compared to non-invaded plots, which will be tested in further analysis. *L. grandiflora* plots had the lowest mean macro-invertebrate abundance (31.33). Some groups were completely absent from invaded plots (Caenidae, Baetidae, Crambidae, Dysticidae, Piscicolidae) while some individuals of these species occurred in adjacent uninvaded plots at the same site.

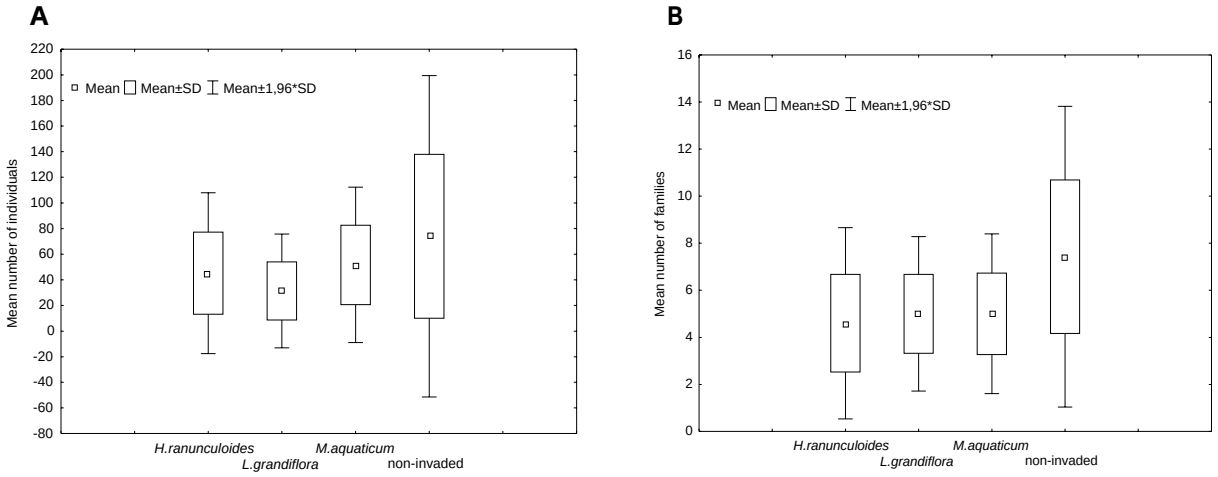


Figure 16: Boxplots showing (**A**) Mean abundance (number of individuals per pond) and (**B**) mean number of families of macro-invertebrates per HIPS and in non-invaded ponds.

Mean 'taxon' diversity (Shannon index) was calculated for each HIPS and for non-invaded ponds, as well as the similarities between them (Morisita-Horn index, software BiodivR, Hardy 2007). No significant differences in faunal diversity could be detected (Table 6A). The Morisita-Horn index shows that invaded ponds are more similar than either one of the HIPS compared to non-invaded ponds (Table 6 B). The analysis of the impact of each HIPS on macro-invertebrate functional groups is in progress.

Table 6: (A) Mean diversity (+/-SD) and (B) mean similarity (both based on families) per HIPS and for non-invaded ponds.

A)

	N taxa	Shannon index
H. ranuncoloides	11	1.03 (0.32)
L. grandiflora	11	1.20 (0.46)
M. aquaticum	8	1.16 (0.25)
Non-invaded ponds	19	1.26 (0.44)

B)

Sample1	Sample2	Morisita-Horn
H. ranuncoloides	*L. grandiflora*	0.920
L. grandiflora	*M. aquaticum*	0.935
H. ranuncoloides	*M. aquaticum*	0.952
H. ranuncoloides	Non-invaded ponds	0.708
M. aquaticum	Non-invaded ponds	0.702
L. grandiflora	Non-invaded ponds	0.683

In 23 water bodies (august 2008), five phytoplankton subsamples were randomly taken in each invaded (B/C plots from Task 1.2) and non-invaded pond (A plots from Task 1.2) and fixed in the field using alkaline lugol, sodium thiosulfate and 4% formaldehyde. Also simultaneously, 10 subsamples of zooplankton were taken in different parts of each pond. The subsamples were combined in the field (total volume 10 L), filtered through a 64 µm mesh net and preserved in 4% formaldehyde. Environmental variables of the water column were measured *in situ* (conductivity, pH, dissolved oxygen, temperature and secchi depth), In each pond type, 10 subsamples of water, taken in different parts of the pond, were pooled into one sample for nutrient analysis: total phosphorus, soluble reactive phosphorus, NH_4^+, (NO_3 + NO_2^-) and chl a were determined in the lab using standard methods (APHA-AWWA-WEF, 1995). Analyses of the samples are in progress.

***Conclusion**: The presence of alien plants and their detritus appears to have a negative impact on macro-invertebrate abundance and number of taxa, with ponds invaded by* L. grandiflora *undergoing the largest decline in abundance. Later identification of phytoplankton and zooplankton will allow us to verify whether the selected HIPS typically influence multiple trophic levels in both water and detritus.*

WP 4 ANALYSIS OF FACTORS THAT MAY MODIFY/ENHANCE HIPS IMPACT ON NATIVE PLANT SPECIES

Two potentially important modifiers of impact are examined experimentally: eutrophication and climate-warming. In both cases we address impact through competition.

Task 4.1.a. Effects of eutrophication on competition between invasive and native species. Terrestrial. (ULB, month 24-48)

In order to prepare the experiment, propagules from three pairs of invaded/native species *(F. japonica with C. arvense, S. inaequidens* with *P. lanceolata* and *S. gigantea* with *E. hirsutum)* have been collected in September and November 2008.

Task 4.1.b. Effects of eutrophication on competition between invasive and native species. Aquatic. (VUB, month 13-18, 25-30, 39)

An experiment was conducted to assess the outcome of competitive interactions between the invasive *Lemna minuta* and the native *Lemna minor*. The plants were grown both in monocultures and mixed communities under constant nutrient levels (Hoagland's solution), light and temperature conditions for 32 days. The *L. minor: L. minuta* planting densities were 0:14, 14:0, 14:14, 14:28, 28:14, 28:28, 0:28, 28:0. The resulting eight planting density combinations were replicated four times yielding a total of 40 beakers (one treatment and four replicates. for the complete design. Dry biomass and frond size were measured at the beginning and at the end of the experiments. The frond size was determined through digital image analysis of photographs of the surface of *Lemna* captured at equidistance from the lens.

The two variables (biomass and frond size) were used to calculate the net relative growth rate between planting and harvesting (RGR, defined as in Connolly and Wayne 1996). RGR's were determined separately for each species using both frond size and plant dry biomass. RGR_i of the i^{th} species equals $\ln(Y_i/y_i)/t$, with y_i and Y_i respectively the initial and final stand dry biomass/pixels of the i^{th} species (i = 1 or 2) and t the duration of the experiment. The relative growth rate difference between the two species was modelled using the Relative Growth Rate Difference (RGRD) model of Connolly and Wayne (1996). RGRD is modelled as: $RGRD = b_0+b_1y_1+b_2y_2+b_3T+\varepsilon$, where b_0 measures the effect of species identity on RGRD, b_1 and b_2 measure the effect of the initial biomass/initial frond area of the first or second species on RGRD, and b_3 measures the effect of treatment on RGRD. "Species identity" refers to the intrinsic difference in relative growth rate between the two species. The RGRD model gives inferences on which species gains (wins) in a mixture given the same/different environmental conditions. This question is concerned with measuring the relative changes in the abundances/proportions of the species over the duration of the experiment. Species proportions in a mixture change when their RGRs differ.

To fit the biomass and frond area in the model, a correlation between the two variables for both *L. minor* and *L. minuta* was tested and a significant positive correlation was observed ($p < 0.05$, $r = 0.87$ for *L. minor* and $r = 0.90$ for *L. minuta*). The variables were fitted into the model separately. In addition, one-way ANOVA of RGR based on biomass with a Tukey's HSD post hoc test (equal variance; $p \geq 0.05$) was calculated to assess the difference in performance of a species, as reflected in its RGR, between monoculture and mixture.

Preliminarily results of the estimated RGRD model based on biomass (Table 8) indicated that species identity (the intercept) and species initial biomass of both *L. minuta* and *L. minor* had a significant effect on RGRD. Effects of species initial frond area, on the other hand, were not significant in the model on Frond area (Table 7). In both models, the intercept (species identity) was significantly different from zero, hence the difference in growth rate reflected by this constant would lead to a change in composition. Both *L. minor* and *L. minuta* generally experienced strong intra and interspecific effects on their RGRs. In the model, a higher initial frond area or biomass of a species caused a relative decrease of the other species final proportions in mixture. The model also predicts that a higher initial biomass of *L. minor (*positive value of b_2 coefficient in Table 7) would enhance the difference in average RGRs in

its favour and hence would tilt the final composition towards it. The negative b_1 has the opposite effect. Results from this model showed that *L. minuta* gained over the period and had a higher average RGR than *L. minor*, measured by the intercept. *L. minuta* relatively gained over *L. minor* in both frond area as well as in biomass.
The ANOVA result on monoculture versus mixture RGR of *L. minor* and *L. minuta* indicated that, at *L. minor*: *L. minuta* densities of 14:0/14:14 and 14:0/14:28, the growth rate of *L. minor* in mixture was significantly lower from that in monoculture ($p<0.001$). In the ANOVA, the RGR of *L. minuta* in mixture was however not significantly different ($p>0.05$) from that in monoculture in all density combinations studied here.

Table 7: Overall estimated models for differences in relative growth rate (RGRD) between *Lemna minor* and *Lemna minuta* over the growth period. Significant values of the t statistics are indicated in bold.

	RGRD Frond area		**RGRD Biomass**	
	R^2= 0.044 $F_{2,17}$ = 0.394 p < 0.68023		**R^2= 0.842** $F_{2,17}$ = 45.263 p < 0.0001	
n = 20	B	t(17)	**B**	**t(17)**
Intercept (species identity)	**0.041128**	**32.45483**	**0.00698**	**3.80782**
Species influence *L. minor* (b_1)	-0.000003	-0.79632	**-5.43476**	**-7.63473**
Species influence *L. minuta* (b_2)	0.000000	0.01942	**3.36761**	**5.21753**

***Conclusion**: In competition experiments, the invasive* L. minuta *relatively gained over the native* L. minor. *Model analysis showed this was due to* L. minuta's *higher relative growth rate (different intercept or "species identity effect"). However, the species initial biomasses also played a significant role in the balance between the species. The next step in this task consists of assessing the influence of different nutrient levels on competition.*

Task 4.2. Effects of climate change on competition between terrestrial and native species. (UA, month 1-30)

In this task we use two species pairs, each consisting of a HIPS with a native competitor (*Senecio inaequidens* with *Plantago lanceolata* and *Solidago gigantea* with *Epilobium hirsutum*). The native competitors were selected from the species with highest cover at the field sites of the INPLANBEL project (33 sites across Flanders, floristic relevés from 2003 and 2004). 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. Containers received optimal water and nutrients to measure only warming effects.
To detect possible pathways of the warming influence, we conducted a combined growth and nitrogen uptake analysis on each type of monoculture and mixed community (Nijs & Impens, 1997). For this purpose, the six replicates per community were harvested at different times during the growing season. At each time, total leaf area was measured, and dry matter separated into roots, leaves and stems. In the mixed communities, invasives and natives were separated above ground, which was not possible for the roots. All plant samples were analysed for N and C concentrations with an element analyser.
The combined growth and nitrogen uptake analysis involved calculations of growth rates, nitrogen uptake rates, and whole-plant nitrogen use efficiency. These were derived from the seasonal courses of measured biomass (B_{above}, B_{below}) and nitrogen concentration ($[N]_{above}$, $[N]_{below}$). These seasonal courses were exponential ($Y = a + be^{c/x}$) for $[N]_{above}$ and $[N]_{below}$, and sigmoid ($Y = b + [(a - b)/(1 + e^{((x - c)/d)})]$) for B_{above} and B_{below}. Further calculations yielded the following derived variables:

- absolute growth rate AGR= $d(B_{tot})/dt$ (where $B_{tot} = B_{above} + B_{below}$)
- relative growth rate RGR= $d(B_{tot})/dt \bullet (1/B_{tot})$
- nitrogen use efficiency NUE= $d(B_{tot})/dt \bullet$ (1/total accumulated N), where total accumulated N = $((B_{above} \bullet [N]_{above}) + (B_{below} \bullet [N]_{below}))$

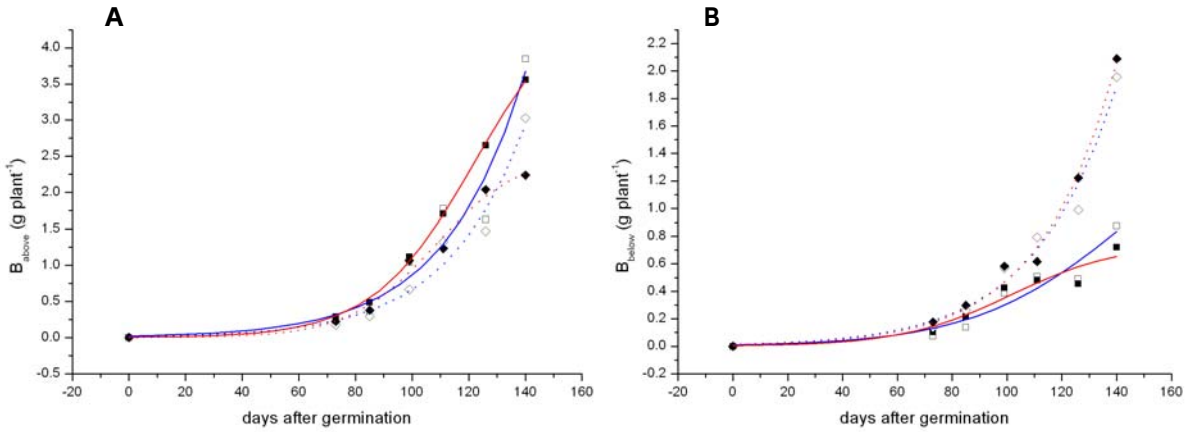
- specific root nitrogen uptake rate, further referred to as specific root activity σ_{root}= d(total accumulated N)/dt • (1/B_{below}).

Computing these variables for all species in the monocultures and the mixtures, as well as in both treatments (heated and unheated), allows to detect how one species can dominate another in ambient climate, how warming effects species in monoculture, and how the competitive outcome is altered by warming.

The measured growth variables (biomass and leaf area) of the species were also analysed with a multifactor analysis of covariance (ANCOVA), with fixed factors origin (native or HIPS) and treatment (climate), and time as a covariate. These analyses were done separately for the monocultures and mixed communities of each species pair.

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 *P. lanceolata*, which was opposite for B_{below} (ANCOVA, $p < 0.05$, Fig. 17 A & B). Together the effects cancelled out in whole-plant biomass (Fig. 17 C). Surprisingly, in spite of its smaller root system, *S. inaequidens* managed to acquire the same amount of nitrogen as *P. lanceolata* (both total nitrogen concentration ($[N]_{tot}$ and total biomass were the same). This was possible owing to a higher root specific uptake activity in *S. inaequidens* than in *P. lanceolata* (Fig. 16 D). The two species used the equal amount of acquired N to produce biomass with the same efficiency (NUE was the same). This too was surprising since, as mentioned above, *S. inaequidens* allocated more biomass to the above ground (i.e. the photosynthetic) compartment. In addition, given its growth habit, *S. inaequidens* probably also had a more efficient spatial display of leaves compared to its rozette-leaved (i.e. self-shading) native counterpart *P. lanceolata*. The latter species apparently compensated for these disadvantages by producing more leaf area (ANCOVA, $p < 0.0001$), ultimately resulting in the same NUE. The two species in this pair thus clearly have different biomass allocation strategies and physiological traits, but, all differences combined they still reached the same productivity in monoculture in the current climate.

We next compare the warming influence on these species, still in monoculture to know the intrinsic effect (or lack thereoff). In both *S. inaequidens* and *P. lanceolata*, warming had no significant effect on B_{tot}, B_{above} or B_{below}. Still, B_{tot} in both species tended to be higher in the heated chambers from +/- day 80, to fall below the values in the unheated chambers again by the end of the season (Fig. 17 C). This trend matches the influence of warming on σ_{root}: in both species, σ_{root} was initially stimulated by warming, to become lower after a peak (Fig. 17 D). We conclude there was little, possibly a small positive, reaction of the monocultures to simulated climate warming, which was similar for both species of the pair.



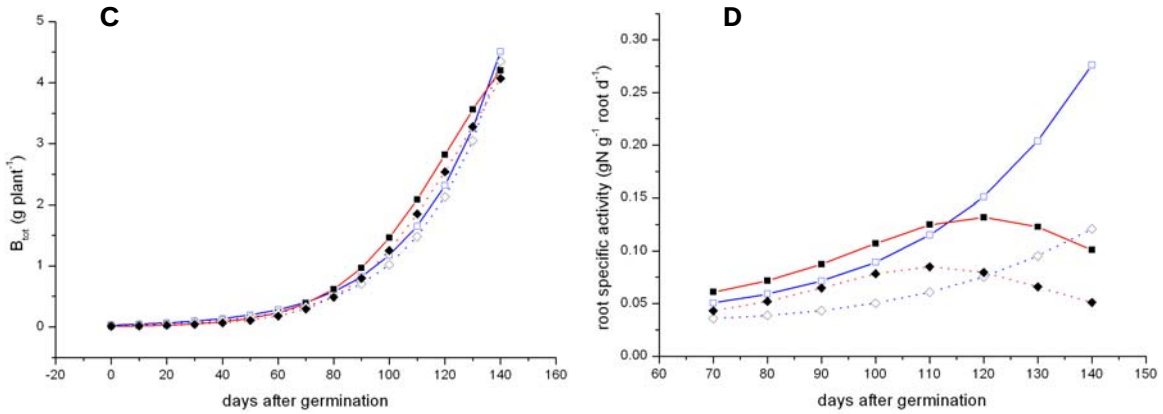


Figure 17: Time courses of aboveground biomass (B_{above})(**A**), belowground biomass (B_{below}) (**B**), whole-plant biomass (B_{tot}) (**C**) and root specific activity (**D**). (A) and (B): measured values and fitted curves (sigmoidal). (C) and (D): curves based on model calculations. Curves for monocultures of *Senecio inaequidens* grown in unheated (□, __) and heated (■, __) sunlit chambers, and monocultures of *Plantago lanceolata* grown in unheated (◊, ...) and heated (◆, ...) chambers.

Also the species of the second pair, the alien *S. gigantea* and the native *E. hirsutum*, showed differences in biomass in ambient conditions in monoculture: B_{above} was significantly higher for *E. hirsutum* (ANCOVA, p = 0.019), while B_{below} was similar (Fig. 18 A & B). Whole-plant biomass was not different (Fig. 18 C). Also this species pair had the same $[N]_{tot}$ (and thus the same total N acquired), this time owing to *E. hirsutum* compensating its lower belowground biomass fraction with a higher σ_{root} (Fig. 18 D). Different biomass allocation strategies and physiological traits thus yielded the same productivity also in this second pair.
Contrary to the first species pair, there was a significant warming effect on the monocultures of the second pair: the future climate decreased B_{below} of the native *E. hirsutum* in the second part of the season (ANCOVA, p = 0.045, Fig. 18 B). This was detectable as a negative trend also in B_{tot} (Fig. 18 C). Belowground biomass of the invasive *S. gigantea*, on the other hand, was unaffected by warming, while B_{above} increased in the second part of the season (ANCOVA, p = 0.021). The root biomass reduction in *E. hirsutum* in the heated chambers coincided with a drastic decline in root specific activity later in the season (Fig. 18 D). As a consequence, N accumulation stopped in *E. hirsutum* at that time, while it continued in *S. gigantea* (not shown). We conclude that, unlike to the first species pair, the monocultures of this second pair showed a different response to the future climate. The native species was set back by impaired root functioning (though not above ground), while the alien species was hardly influenced.

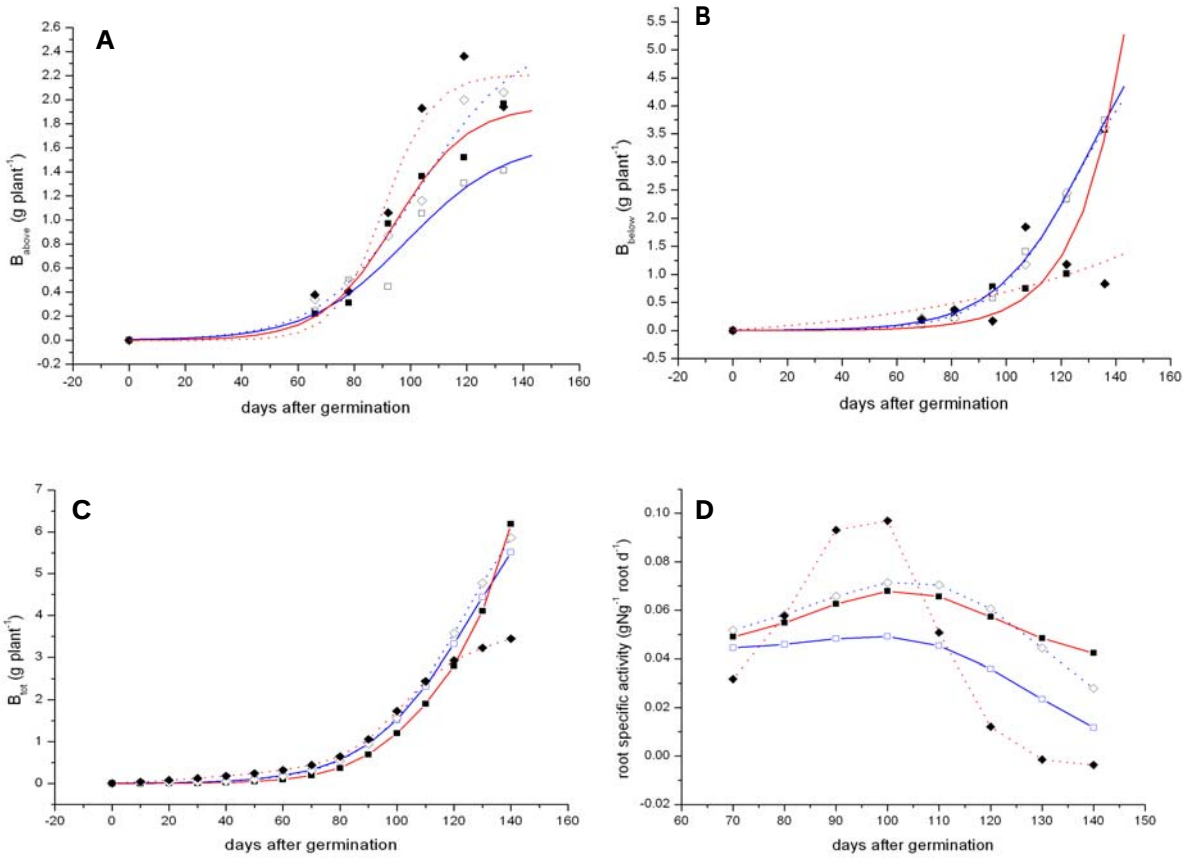


Figure 18: Time courses of aboveground biomass (B_{above}) (**A**), belowground biomass (B_{below}) (**B**), whole-plant biomass (B_{tot}) (**C**) and root specific activity (**D**). (A) and (B): measured values per plant together with fitted curves (sigmoidal). (C) and (D): curves based on model calculations. Curves are shown for monocultures of *Solidago gigantea* grown in unheated (□, __) and heated (■, __) sunlit chambers, and monocultures of *Epilobium hirsutum* grown in unheated (◇, ...) and heated (◆, ...) chambers.

With the growth strategies of the species and their responses to warming analysed, we can now turn to the enhanced complexity of competing species and ultimately to the influence of warming on competition. We first compare monocultures with mixed communities in ambient climate (above ground, as roots could not be separated). When the species of the first pair were grown together, the alien *S. inaequidens* supressed the native *P. lanceolata* (ANCOVA, significant origin effect for mixed communities, p = 0.034, Fig. 19 A). While this was according to expectation based on the monocultures, where *S. inaequidens* was indeed more productive (Fig. 17 A), growing together strongly amplified the monoculture difference (*P. lanceolata* lost 49% of its monoculture biomass, *S. inaequidens* gained about 33%). The biomass difference between the species in the mixture was reflected in their total acquired nitrogen (Fig. 20), suggesting that the alien *S. inaequidens* took away nutrients from its native competitor. The cause of this is clear from Fig. 17 D: the alien is a much better competitor for nitrogen given its much greater σ_{root}. Competition above ground may have amplified this difference in below ground physiological traits, but was not the cause since the species had the same NUE (see above).

When the species of the second pair were grown together, the native *E. hirsutum* "won" the competition (=Fig. 19 B, note: above ground biomass). However, although *E. hirsutum* became about 40% more productive in mixture than in monoculture, *S. gigantea* remained unaffected by its competitor (Fig. 18 A and Fig. 19 B). The same pattern was found in the time course of above ground accumulated N (not shown). Also in this second species pair, the dominant species had the higher σ_{root} (Fig. 18 D), while the NUEs were not different. The mechanism was not entirely the same as in the first pair though, since *E. hirsutum* acquired part of its nitrogen from additional sources: the mixture acquired more nitrogen than the

average of both species separately. This points to complementary resource exploitation, be it that only one species profited.

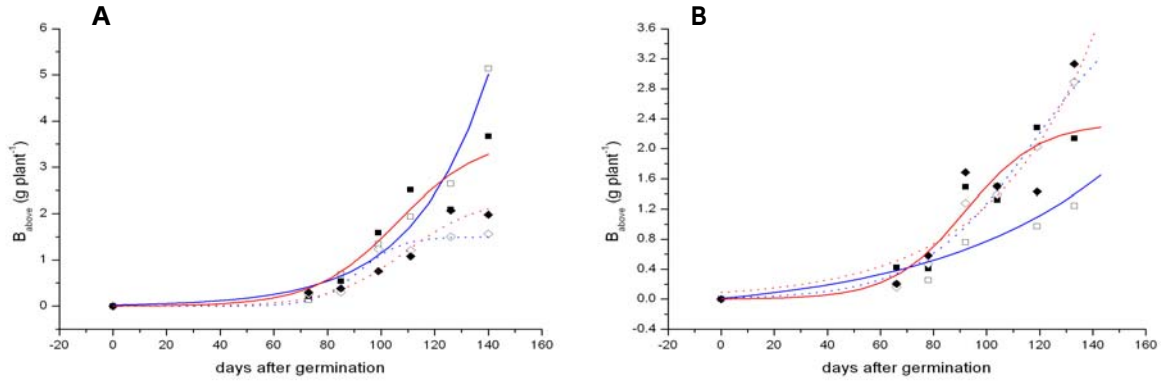


Figure 19: Time courses of aboveground biomass (B_{above}) for mixed communities of species pair *Senecio inaequidens – Plantago lanceolata* (**A**) and species pair *Solidago gigantea – Epilobium hirsutum* (**B**). Measured values per plant and fitted curves for the alien species of the pair grown in unheated (□, __) and heated (■, __) sunlit chambers, and the native species of the pair grown in unheated (◊, ...) and heated (◆, ...) chambers.

How did the simulated warming modify the outcome of competition? In the mixtures of the first species pair, by the end of the season a negative respons in B_{above} of the alien *S. inaequidens* and a positive respons of the native *P. lanceolata*, had greatly decreased the above ground advantage of the alien found in ambient conditions (Fig. 19 A). In the future climate, *S. inaequidens* produced only 85% more than *P. lanceolata*, as opposed to 229% more in the current climate. Fig. 18 D suggests that this biomass convergence between the competitors under warming originated from σ_{root} values becoming more similar.
A contrasting result was found for the second species pair, in the sense that here the alien species *S. gigantea* was stimulated by the warmer conditions, while B_{above} of the native *E. hirsutum* remained unaffected (ANCOVA, significant treatment x origin effect, p = 0.044). As a result, the 57% advantage of the native over the alien species observed in this pair in ambient conditions, was reduced to only 31% in a warmer climate (Fig. 19 B). The similarity with the first pair is that these responses likewise lead to convergence of above ground biomass. The positive effect of warming on *S. gigantea* can again be traced to root specific activity (increased σ_{root} under warming throughout the season, Fig. 18 D).

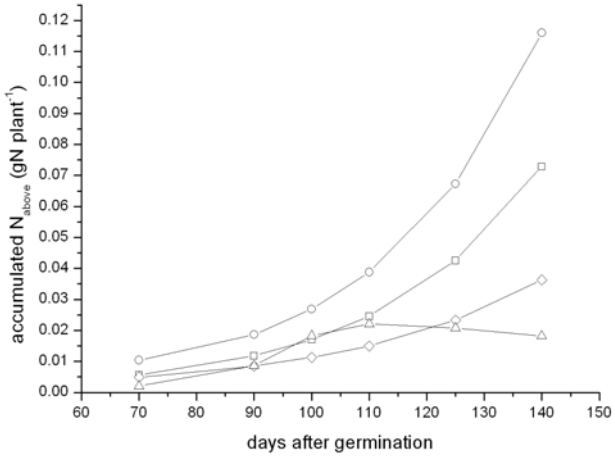


Figure 20: Time courses of aboveground accumulated nitrogen. Curves based on model calculations for *Senecio inaequidens* grown in monocultures (□) and mixed communities (○) and for *Plantago lanceolata* grown in monocultures (◊) and mixed communities (Δ), all in unheated chambers.

A second experiment, in which we investigate whether warming and associated changes in water availability will affect competition, has been conducted (summer 2008). This time, three HIPS were used (*S.gigantea*, *S. inaequidens*, *F. japonica*). In a similar experimental set-up, all containers received natural precipitation, to allow for summer drought. During the growing season plants were screened for a series of ecophysiological characteristics to assess whether changes in temperature and water availability systematically favoured the invasive species. Additionally, symptoms of drought stress were examined with chlorophyll fluorescence. Plants were harvested at the end of the growing season for biomass determination. Data analysis is in progress.

***Conclusion**: Of the two species pairs tested, in one pair the HIPS dominated in mixture, and in the other pair the native species. Climate warming in the mixtures reduced the HIPS dominance in the first species pair, but stimulated the suppressed HIPS in the second. Most of these responses could be traced to root specific nitrogen uptake capacity. Responses of above ground biomass to warming in monoculture were not always a good predictor of responses in mixture.*

4. PRELIMINARY CONCLUSIONS AND RECOMMENDATIONS

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

In terrestrial communities, plant species richness loss was a common trend associated with invasion for *Fallopia* spp., *I. glandulifera* and *S. gigantea*, but not for *S. inaequidens*. Impact increased with HIPS density except for *I. glandulifera*. It would thus be possible to prioritize HIPS for control measures depending on species. 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 impact 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, our (preliminary) assessment at this time is that presence of HIPS in nature reserves would rather be 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. No indicator species were associated to the invaded vegetation, except the HIPS themselves, but further analysis may perhaps reveal indicator functional groups. In spite of the severe diversity loss induced by most HIPS, our results did not confirm the generally accepted hypothesis of plant communities homogenisation, except for *Solidago gigantea*. β-diversity thus seems less affected than α-diversity, which may become an element in finetuning control measures (e.g. invest in landscape design measures or local control 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 (and to a lesser extent emergent macrophytes). This knowledge can help select invaded ponds for control: ponds with those growth forms may 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 both, rather than a strong focus on either type. Chlorophyll a and alien cover were negatively correlated which suggests that aquatic HIPS also have less visible impact such as phytoplankton decline. The severity of this impact will be examined further, which may likewise help setting priorities.
Please note that the above recommendations are preliminary!

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 value of N fluxes into aboveground organs, and a high N resorption efficiency from senescing shoots. Superior N use and production of recalcitrant litter stand out as two major components of the impacts of *F. japonica* invasion. Both traits may confer a high competitive superiority. The data suggest that soil impact may last after *F. japonica* is removed, possibly even requiring topsoil removal to restore previously invaded sites after control. Decomposition rates in invaded plots were slightly higher, which may be explained by *F. japonica* actively modifying the microclimate under its canopy. Possibly the dense canopy of *F. japonica* increases relative air humidity close to the surface, decreasing evaporation. Microclimate data have to be collected in order to confirm this additional impact pathway on decomposition.
In the future, it should be interesting to build a model explaining N and C fluxes between all different organs of *F. japonica* and soil in order to fully understand the impact of this species on C and N cycling, and its repercussions for outcompeting native species and for restoration of infested sites.
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. The observed increase in P pools of belowground organs is due to both increased biomass and increased P concentrations in invaded plots. 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.*
The hypothesis of a positive feedback of *F. japonica* on its own competitive success by the modification of soil properties has not been verified. Indeed, no significant difference was observed between plant performance in invaded and uninvaded soils. On the other hand, in the invaded soil amended with charcoal, plant performance was different. In invaded and uninvaded soil, *C. arvense* seemed to grow better in pure culture while it grew better in mixed culture in soil amended with charcoal. In other words, it "loses" the competition with *F. japonica* in invaded and uninvaded soil while it "wins" in amended soil. This indicates that the competitive superiority of *F. japonica* is probably partially due to allelopathic properties since it loses this superiority in the presence of charcoal.
Pollinator-mediated impacts of HIPS on native species vary between alien species and pairs of native – invasive species. Preliminary results did not show negative impacts of HIPS on the reproductive success of native species. There were effects, however, on the visitor guild, the number of visitors and on pollinator foraging behaviour, which were often proportional to the number of HIPS. The effects became weaker at greater distance.

Effects of HIPS can strongly proliferate to other trophic levels

In terrestrial systems, we demonstrated a dramatic decline in soil fauna abundance under *F. japonica*. Although there was no change in diversity, also the soil fauna assemblages were significantly altered. Reduced habitat heterogeneity and resource diversity in *F. japonica* stands may explain the observed decline in the total number of individuals of the soil fauna. Altered microclimatic conditions might, in part, explain shifts in faunal assemblages.
In aquatic systems, preliminary analysis suggested an impact on the structure of the macro-invertebrate community, which requires further investigation. Ponds invaded with *L. grandiflora* suffered the most in terms of loss of both macro-invertebrate abundance and diversity. After determination of phytoplankton and zooplankton samples we will be able to ascertain whether effects can be generalised to more trophic levels.
Our preliminary conclusion is that loss of biota due to HIPS invasions is probably larger than can be estimated from the effects on native plant communities alone.

Which factors modify impact?

Data to characterize a possible enhancing or mitigating influence of eutrophication on impact are not yet available as they follow later in the project (nutrient addition series experiments in aquatic and terrestrial systems). In a preparatory experiment for aquatic systems, the competitive superiority of the invasive *L. minuta* over the native *L. minor* has been established experimentally and explained with a competition model. This will help interpret the later work on impact of eutrophication on competition in this system.
Simulated climate warming clearly modified current competitive interactions between native and invasive terrestrial plants. However, the way in which the balance between HIPS and native species was altered, depended on the studied species pair: in one pair the invasive species was favoured, in the other pair the native species. These changes could be ascribed to warming influences on nutrient uptake, again confirming the importance of belowground processes. From the species pairs examined, it appears that the sensitivity of the native-invasive interaction to climate warming does not necessarily mirror the intrinsic (monoculture) sensitivities of the species.

Overall, our results up to now support that HIPS do more to ecosystems than merely suppress native competitors. A wide range of HIPS impacts exist, both in terrestrial and aquatic systems, and a number of these are severe. Impacts further in the foodweb are not necessarily weak, and the soil is a key compartment for understanding impacts. The data also provide experimental support for the widespread hypothesis that climate change is likely to alter alien plant invasions.

5. PROSPECTS AND PLANNING FOR PHASE 2

WP 2 Mechanisms of HIPS impact on native plants

Task 2.1. Elucidating direct impact via niche overlap (UA, month 27-42)

Objective: Assess whether niche overlap can explain the outcome of native-invasive competition at microscale.
Experimental design: Field study on HIPS *Senecio inaequidens, Prunus serotina, Solidago gigantea, Fallopia japonica, Rosa rugosa*, and *Impatiens glandulifera* (same sites as Tasks 1.1 and 2.3):
(i) are microsites (gaps) where invaders occur, surrounded by neighbour species with different traits than microsites where no invaders occur? (sites at different distance from the invader front);
(ii) for microsites where invaders occur: do invaders grow more vigourously where they are surrounded by native neighbours with different traits than the invader, or, conversely, by native neighbours with similar traits? Assumption: species with similar traits share the same niche.
(iii) Does invader impact on its native neighbours depend on trait overlap?
Data collection and analysis: We will sample traits related to niche differentiation along axes of light, nutrient, and water availability: specific leaf area (SLA), leaf light-saturated photosynthetic rate (P_{max}), dark respiration rate (R_d), photosynthetic compensation irradiance (I_0), apparent quantum efficiency (QE), photosynthetic nitrogen use efficiency (PNUE), photosynthetic water use efficiency (PWUE), leaf chlorofyll concentration ([Chl]), leaf nitrogen concentration ([N]), root/shoot ratio (R/S), leaf mass ratio (LMR). For example, if both the invader and its neighbours are shade-tolerant species, they are likely to share high values of SLA, QE, LMR and low values of P_{max}, R_d, I_0, [Chl], [N], and R/S.
To analyse question (i) we apply discriminant analysis. For questions (ii) and (iii), convergence (or divergence) between species is expressed as Euclidian distance in multivariate trait space. The spatial scale at which the interactions occur (neighbourhood size of invader) will be analysed with multiple regression analysis as in Milbau *et al*. (2007).

Task 2.2. Elucidating indirect impact mediated by pollinators.

Task 2.2.a Elucidating indirect impact mediated by pollinators for terrestrial systems (FUSAGx & UCL, month 13-42)

Objective: Assess whether HIPS affect reproductive success of native plant species and whether those impacts are mediated through modification of pollinator services. Selected HIPS are *S. inaequidens*, *I. glandulifera, Fallopia* spp. and *Solidago* spp.

Subtask 1: Modification of reproductive success of selected native competitors by *Fallopia* spp. and Solidago gigantea, field approach

Experimental design: In this task, we will complement our knowledge on HIPS impact on native reproductive success in field situation for the two species for which no data are currently available. This analysis will largely mirror the results obtained by partner 5 on *S. inaequidens* and data form literature on *I. glandulifera*. Once native competitors will be selected using a food web approach, one site/HIPS, where the two species are found in sympatry, will be selected. This subtask will not be performed for *I. glandulifera* given the fact that field experiments are already well documented for this species.
Data collection and analysis: In each site 12 individuals of each native species will be selected along a density gradient for non-clonal HIPS or at different distances from the clones' front for clonal HIPS. The reproductive success and the pollination components of each individual will be assessed. For reproductive success we will measure fruit set / seed set / fertilization rate / abortion rate, at various times of the flowering period of individuals. Assessment of pollination services will be based on the following observations during the same

period of flowering: during a fixed period of time (15 or 30 min spread over the day with two observers working together, alternatively we will test video monitoring), recording all insects visiting selected individuals of native target species and adjacent individuals of HIPS (discriminating functional groups: bumblebees, syrphids,….); capturing visitors for further determination and assessment of the quantity of native/exotic pollen transported; collecting stigma of native species individuals for assessment of quantity of native/exotic pollen deposit. For each individual of native species, the following parameters will be measured: mean distance to the three nearest HIPS neighbours or distance to the clone front, density of HIPS individuals in 1.5 m radius, mean distance to the three conspecific individuals, density of conspecific individuals in 1.5 m radius, relevés of all native species present in a 1.5 m radius with their percentage cover. Multivariate analysis (best subset multiple regression) will be used to assess the relative influence of HIPS (mean neighbour distance, density) and native communities (mean distance to and density of conspecific native, native species richness and diversity, index of native communities composition obtained from PCA ordination) on the different components of reproductive success. Analysis will be conducted for all sites together and for each site individually to test for idiosyncratic effects. We will control for pollination to disentangle the effect of competition for resources and pollinators services. Pollen addition experiments (corresponding to maximum potential pollination) will be conducted on a set of flowers on each individual and the reproductive success will be compared with freely pollinated flowers. In case reproductive success in the native species is still affected by HIPS density when pollination is controlled, this would indicate the part of reproductive success modification due to competition for resources as compared to competition for pollination.

Subtask 2: controlled experiments to assess alteration of pollinator services

Validation and publication of results require experimentations during at least two consecutive years. Therefore controlled competition experiments in experimental parcels with invasive-native pairs (*S. inaequidens – S. jacobaeae*; *I. glandulifera – E. angustifolium*; *I. glandulifera – A. napellus*) started the second year of phase 1 and will be repeated during this second phase. If necessary, some parameters (e.g. distances, HIPS densities, number of natives, etc.) could be adapted respect to our experience and first data acquired during months 13-24. When a relevant native competitor is found for *Fallopia* spp. or/and *Solidago* spp. during months 13-24 (Substak 1), this experiment could also be realized with one of these HIPS.

Task 2.2.b Elucidating indirect impact mediated by pollinators for aquatic systems (VUB, month 31-35)

Objective: Track pollinator movements at short distance classes and explore within- and between-population pollen dispersal patterns along an invaded and non-invaded plot
Experimental design: As case study we will examine the pollen flow within infestations of the flower-rich *Ludwigia*, within adjacent native populations of insect-pollinated plants, and between both. A non-invaded plot is taken as control. Additionally, we measure over which distance the pollinators potentially move pollen and whether the size of the exotic population matters. We will use fluorescent powdered dye, which is considered a good analogue of pollen, to track pollinator movements and estimate the spatial pattern of pollen dispersal (Young, 2002). This method was applied by partner 3 in a previous project.
Data collection and analysis: Fluorescent dye of different colours will be deposited on stamens of donor plants and after two days stigmas of recipient plants are collected and mounted on slides. The presence/absence of dye on recipient plants allow to test for the potential positive or negative effect on native plants, mediated by pollinators.

Task 2.3.b Impacts of soil modifications on competition between native and alien species (ULB, month 7-30)

Objective: Test if soil alteration by HIPS modifies competitive balance between HIPS and native species at the advantage of HIPS and may thus result in a positive feedback.

Experimental strategy: A competition experiment between *F. japonica* and a native species (*C. arvense*) already started during phase 1. Two pure cultures and a 50:50 mixture are growing in pots in three soil treatments (soil from invaded plots, soil from uninvaded plots, soil from invaded plots and amended with charcoal).
Data collection and analysis: We decided to extend this study to month 36 in order to monitor growth for two years. During those two years, non destructive measurements (number of leaves axes length (main stem + ramifications), length and width of the largest leave) will be made at 3-month intervals (starting from June 2008). Data will be analysed using fixed ANOVA. Enhanced competitive ability of *F. japonica* on its own soil will point to positive feedback effects. Difference in competitive ability between charcoal amended and unamended soil will point to allelochemicals-mediated effects.

WP 3 Impacts at other trophic levels

Task 3.1 Terrestrial: Impacts of HIPS on soil fauna (ULB, month 13-36)

Objective: Test if HIPS that alter topsoil mineral element composition also modify animal communities in and above soil.
Experimental design: This task includes four HIPS and two of them (*F. japonica* and *S. gigantea*) were studied during phase 1. Two other HIPS, *S. inaequidens* and *I. glandulifera* will be studied during phase 2. Four sites per species will be chosen among the sites selected by partner 2 (task 1.1.). At each site, soil fauna will be sampled from 12 plots. These will be organized along a gradient of invasive species cover. Alternatively, in case of sharp boundary between invasive species stands and native vegetation, 6 plots will be located in invaded stands and 6 others in adjacent, uninvaded stands. Volumetric soil cores will be collected (depth: 0-10 cm). Soil microarthropods will be extracted from the fresh soil cores by a modified Berlèse-Tullgren funnel method. Epigaeic fauna will be collected by pitfall traps. For earthworms, a turf 10 cm deep and the width of a spade is cut. The sample is broken up on a tray and the worms are collected.
Data collection and analysis: Microarthropods will be sorted into main groups of Collembola, Oribatid mites and non Oribatid mites. The macroarthropods of the pitfall traps will be sorted into broad taxonomic groups and weighed. Earthworms will be sorted to species and weighed t-tests and chi-square tests will be applied to compare density and taxonomic assemblages between invaded and uninvaded plots.

Task 3.2 Aquatic: Impact of HIPS on other trophic levels in water/sediment (VUB, month 19-24, 36-38)

Statistical analysis of abundance, diversity and functional groups will be performed for phytoplankton, zooplankton and maco-invertebrate data with uni-and multivariate methods (month 36-38).
Preliminary results show that invaded ponds seem to have an effect on macrophyte abundance and community structure. After identification of all phytoplankton and zooplankton we will verify whether this effect is pronounced at different trophic levels in both water and detritus. Multivariate analysis revealed a negative correlation between chl a (proxy for phytoplankton biomass) and HIPS cover. The latter suggests an impact of HIPS cover on phytoplankton abundance and composition. This hypothesis will be further tested at phytoplankton division level.

WP 4 Analysis of factors that may modify/enhance HIPS impact on native plant species

Task 4.1 Effects of eutrophication on competition between invasive and native species

Task 4.1.a Terrestrial (ULB, months 24-48)

Objective: Test if soil eutrophication enhances competitive advantage of HIPS over native species

Experimental design: A competition experiment will be conducted in pots with four soil treatments representing a soil fertility gradient (Nitrate, ammonium and P added as soluble salts; the highest level will represent the yearly N-P dressing currently used in intensive agriculture). Target HIPS are *F. japonica, S. gigantea, S. inaequidens*. The native competitor will be taken from the dominant species in the native vegetation at the respective natural sites. The design of the competition experiment will be the same as for Task 4.2.
Data collection and analysis: Non destructive measurements will be made at 3-month intervals (shoot height, number of leaves) and calibrated in terms of biomass by linear regression. The kinetics of competition for nitrogen will be examined by determining nitrogen content of a single, standardised leaf harvested at each date (correlation between N content of single leaves and whole plants will be calibrated on an extra set of plants that will be destructively sampled). The plants (above and, if possible, belowground parts) will be harvested after 2 years, weighed and analysed for N and P content. Protocols for data analysis and interpretation are as in task 4.2.

Task 4.1.b Aquatic (VUB, month 13-18, 25-30, 39)

Objective: Test if eutrophication enhances competitive advantage of HIPS over natives and if eutrophication favours one out of two competing invader species.
Experimental design & data collection and analysis : Two growth forms were selected after initial trials: free-floating leaved 'lemnids' and submerged rooted 'elodeids'. A first competition experiment between native *Lemna minor* and invasive *Lemna minuta* was conducted under similar eutrophic conditions in months 13-18. A second competition experiment between submerged HIPS is ongoing. The direct competition capacities under similar nutrient conditions between *Egeria densa* and L*agarosiphon major* will be estimated. Those recent 'elodeid' HIPS will be investigated as was published earlier on *Elodea nuttalli* vs *L. major* (James *et al*., 1999) or *Elodea canadensis* vs *E. nuttalli* (Barrat-Segretain *et al*., 2004). We actually test the competitive ability and subsequently will select the invasive species that will be used in competition experiments against a native species (easy manipulation, growth structure...) under two abiotic conditions (month 25-30, 39). The abiotic conditions represent sediments taken from 1) an unmanaged pond with a high amount of organic sediments 2) a restored pond ecosystem – removal of upper layer- with a lower amount of nutrients in the remnant soil.

Task 4.2 : Effects of climate change on competition between terrestrial invasive and native species (partner 1: months 1-30)

Objective: Assess whether climate change modifies native-invasive interactions (i) systematically (e.g. consistent competitive shift towards invasive species), (ii) ideosyncratically (unpredictable changes), or (iii) not at all (no shifts). Can observed patterns be explained by different ecophysiological responses to the warming in invasives and natives, and do changes in water availability associated with warming affect the invasive species differently?
Experimental design & data collection and analysis: During phase 1, 2 competition experiments were conducted where species pairs (HIPS with a native competitor) were exposed together with all possible monocultures to experimentally induced temperature increase in climate-controlled greenhouses. In year 1 all communities were supplied with optimal water and nutrients, to assess the intrinsic response to warming. In year 2 the stands only received natural precipitation, to allow summer drought.
In both experiments, the species were screened for morphological and ecophysiological characteristics that are potentially related to interspecific variation in growth rate. In year 1 an additional combined growth and nitrogen uptake analysis was conducted, in year 2 effects of drought were examined additionally with chlorophyll fluorescence (F_v/F_m) as a stress indicator. Responses of natives vs. invasives are tested with ANOVA.
During phase 2 (month 25-30) data analysis of experiment 2 will be completed and published.

WP 5 Integration (all partners) (months 36-48)

Integration will be achieved by the structure of the project: shared species, landscapes, and experimental protocols, and mirror experiments for terrestrial and aquatic HIPS. This makes it possible (a) to disentangle impact pathways and compare impact at different spatial scales, (b) to conduct combined analyses on data from different WPs.

(a) Integration across scales and organizational levels. The use of 4 core species (*S. gigantea, F. japonica, S. inaequidens, I. glandulifera*) in a shared landscape (Kessel) is common to Tasks 1.1, 2.1, 2.2.a, 3.1, and 4.2. This allows quantitative comparison of the impacts of the same HIPS **at different levels of ecological organisation**: direct impact on native competitors (2.1 and control treatment of 4.2), indirect impact via pollinators (2.2.a), impact on soil fauna (3.1). It also allows comparison of HIPS impact on native plant diversity **at 3 spatial scales**: microscale (2.1, control treatment of 4.2), habitat patch scale, and landscape scale (both in 1.1). The tasks on modifiers of impact (4.1 and 4.2) have 3 species of the core set in common (*S. gigantea, F. japonica, S. inaequidens*) and share the same protocol. The terrestrial/aquatic comparison of impact will be made for different spatial scales (large scale in 1.1 vs. 1.2, small scale in control treatments of 4.1.a vs. 4.1.b), but also for indirect impact via pollinators (2.2.a vs. 2.2.b) and impact at higher trophic levels (3.1 vs. 3.2), and for effects of eutrophication (4.1a vs. 4.1b).
This built-in commonality in the experiments allows disentangling of impacts operating through different pathways.

b) Integrative analyses on combined data from different WPs
(i) Do invaders that strongly displace natives in pots (control treatments of Task 4.1 and 4.2) have large impacts on native plant diversity in the field?: (partners 1, 2 and 4)
(ii) Does niche-overlap measured in the field (Task 2.1) and on the species-pairs in controlled conditions (Task 4.2, control treatment) similarly predicts invader impact? (partner 1)
(iii) Do species exhibiting the greatest impacts (on soil biota, local plant diversity,...) also have the widest range in habitat selection (Task 1.1) or in expansion rate (available from INPLANBEL)? If so, which impact correlates best with range or rate? (partners 2, 4, 5)
(iv) Additional analyses with direct value for users: see Task 6.1.
(v) Conceptual integration (cf. figure above) of impact pathways by aquatic invaders (partner 3).
Note: as partner 5, who provides the specific expertise on pollination, will be involved only in years 2 and 3, integration will be assured through partner 4, who will work on the same tasks.

WP 6 Valorisation (all partners) (months 1-48)

Task 6.1 : Products and services (all partners: months 6-48)

(i) **Checklist** of which native communities are affected most by HIPS. For example, high or low-diverse? Composed of species with low or high Ellenberg numbers for N (link with eutrophication in Task 4.1) ? Composed of which life-forms or C-S-R strategies? Derived from the landscape surveys on biodiversity impact in Tasks 1.1 and 1.2. (partners 3 and 4)
(ii) Do **bio-indicators** exist for impact of invasive species: which native species (plant or animal) are highly sensitive, i.e. decline most in the community, or disappear first with increasing HIPS density? Derived from 1.1 and 1.2 (partners 3 and 4), and from 3.1 and 3.2 (partners 2 and 3).
(iii) **Integration paper**. The partners commit to jointly write and submit for publication a paper in which the data of the project are combined, and the pathways disentangled (cf. WP 5). (all partners, lead: partner 1)
(iv) Guided **field tours** for the public and local actors of in situ impact of HIPS on native biodiversity (partners 4 and 5).

Task 6.2 Follow-up committee meetings (all partners, month 1-48)

Four follow-up committee meetings will be held during phase 2.

6. PUBLICATIONS / VALORISATION

6.1. Publications of the teams

6.1.1. Peer review

UA

Verlinden M, Nijs I. Experimental climate warming favours alien plant species over congeneric natives. Submitted to New Phytologist.

ULB

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.

Domken S, Dassonville N, Meerts P, Josens G. Impact of the exotic invasive plant species *Fallopia japonica* on soil fauna and litter decomposition. Submitted to Plant and Soil.

FUSAGx

Piqueray J, Mahy G, Vanderhoeven S. Naturalization of a horticultural species, *Cotoneaster horizontalis* DECAISNE (Rosaceae) in biodiversity hotspots in Belgium. Belgian Journal of Botany. Accepted.

6.1.2. Others

UA

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 and 22 May 2007. Poster 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.

Milbau A, Stout JC & Nijs I (2007). Which traits promote successful establishment and spread? From native colonizers to alien invaders. Abstracts of the international workshop 'Colonization versus invasion: do the same traits matter?', Ascona, Switzerland, 25 February – 2 March 2007, p. 29.

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).

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.

VUB

Stiers I, Triest L : " The impact of invasive alien plant species on species diversity in aquatic communities.", Symposium Starters in Nature Research, Brussels, 20 March 2008. Poster Presentation.

Stiers I, Triest L: "The impact of invasive alien plant species on species diversity in aquatic communities", Conference Plant Population Biology for the coming decade, Luxembourg, 1-3 May 2008. Poster Presentation.

Science popularisation : " Heb je ook exoten in huis ? / Avez-vous aussi des exotiques chez vous ? ", Milieufeest / Fête de l'environnement 1 June 2008, Brussels, Participation with poster and pamflet.

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

FUSAGx
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 and 22 May 2007. Poster presentation.

UCL
Senterre M. 2009. (In french). Impact of invasive species *Impatiens glandulifera*, on pollination of two native species *Epilobium angustifolium* and *Aconitum napellus* subsp. l*usitanicum*. M.Sc. thesis, Faculté des Sciences, UCL, Belgium, 85 p.

Cawoy V, Senterre M, Jacquemart AL. 2008. 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.

Mahaux O. 2008. (In french). Biology of the reproduction and interactions plants – pollinators: comparison of an invasive species with his native congeneric native species. M.Sc. thesis, Faculté des Sciences, UCL, Belgium, 72 p.

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

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

Vervoort A, Jacquemart AL. 2007. (In French). Problems with invasive species, an example with *Impatiens* spp. Forêt wallonne 91: 10-17.

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

6.2. Co-publications

6.2.1. Peer review

ULB & UCL
Vanparys V, Meerts P, Jacquemart AL. 2008. Pollinators and reproductive success in *Senecio inaequidens* and *S. jacobea*. Acta Oecologia 34: 361-369.

6.2.2. Others

ULB & UCL

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

6.3 Other activities

Contribution to the Belgian Biodiversity Platform (http://ias.biodiversity.be/), part aquatic invasive species in Belgium (by VUB), and part on *Impatiens parviflora* (by UCL).

Project Website: http://www.ua.ac.be/alienimpact

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

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Milbau A, Reheul D, De Cauwer B, Nijs I. 2007. Factors determining plant-neighbour interactions on different spatial scales in young species-rich grassland communities. Ecological research 22: 242-247.

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