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Vnitrodruhová zpětná vazba mezi rostlinou a půdou jako mechanismus invazivnosti neofytů České republiky

Intraspecific plant-soil feedback as a mechanism underlying invasiveness of neophytes of the Czech Republic

Diplomová práce

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Prohlášení:

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V Praze,

Podpis

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## **ABSTRAKT**

Vnitrodruhová zpětná vazba mezi rostlinou a půdou je vztah, ve kterém druh ovlivňuje složení půdy, a tyto změny v půdě zpětně ovlivňují stejný rostlinný druh. Tento vztah a jeho intenzita může být spojena s dominancí rostlin a jejich invazivitou. Dominantní druhy mohou měnit složení půdy ve svůj prospěch a tak zažívat pozitivní vnitrodruhovou zpětnou vazbu. Vzhledem k tomu, že invazní druhy jsou v novém prostředí obvykle dominantní, dá se očekávat, že pozitivní zpětná vazba s půdou může být důležitým faktorem umožňujícím druhům stát se invazními.

Abych zjistila, zda li vnitrodruhová zpětná vazba mezi rostlinou a půdou může být faktorem podmiňujícím invazivnost rostlin, porovnála jsem zpětnou vazbu s půdou ve skupině invazních a naturalizovaných, ale neinvazních, rostlin České republiky. K realizaci jsem použila před vybraný soubor 34 druhů – 17 invazních a 17 neinvazních. V rámci pokusu jsem použila metodu dvoufázového experimentu. V první fázi dochází k ovlivňování půdy určitým druhem. Ve druhé fázi je stejný rostlinný druh pěstován v ovlivněné půdě z první fáze a jako kontrola je použita půda neovlivněná žádným druhem. Poté je porovnána rostlinná biomasa z ovlivněné a kontrolní půdy.

Výsledky naznačují, že invazní druhy vykazují více pozitivní nebo alespoň méně negativní vnitrodruhovou zpětnou vazbu mezi rostlinou a půdou než neinvazním druhům. To znamená, že vnitrodruhová zpětná vazba mezi rostlinou a půdou může být mechanismem podmiňujícím invazivnost rostlin. Výsledky této práce nám mohou pomoci pochopit mechanismy invazivnosti rostlin a tudíž bude možné lépe předpovědět, které druhy jsou potenciálně invazivní.

**Klíčová slova:** vnitrodruhová zpětná vazba mezi rostlinou a půdou, rostlinné vlastnosti, invazní rostliny, neofyty České republiky

## **ABSTRACT**

Intraspecific plant-soil feedback is a relationship in which plant affects the composition of the soil and such modified soil affects growth of the same plant species. This relationship and its intensity may be linked with plant dominance and invasiveness. Dominant species can alter the composition of the soil in their favor and thus show positive intraspecific plant-soil feedback.

As the invasive species are commonly being dominant in their new environment, it can be expected that intraspecific positive plant-soil feedback could be an important factor allowing the invasive species to achieve their dominant position and become invasive.

To test if the existence of positive intraspecific feedback could be a general mechanism underlying plant invasiveness I compared intraspecific plant-soil feedback in a group of invasive and introduced, but non-invasive, plants in the Czech Republic. I did this using a preselected set of 34 species - 17 invasive and 17 non-invasive. For realization of the project I used the method of two-phase experiment. The first phase is called soil conditioning – influencing of soil by the plant. In the second phase the same plant species are planted in conditioned soil from the first phase and in control (unconditioned) soil. Then I compared plant biomass from conditioned and unconditioned soil.

The results suggest that invasive species show more positive or at least less negative intraspecific plant-soil feedback than non-invasive species. It means that intraspecific plant-soil feedback could be the mechanism underlying plant invasiveness. These results may improve our understanding of invasive mechanism of plants and thus we can better predict which species are potential invasive.

**Key words:** intraspecific plant-soil feedback, plant traits, invasive plants, neophytes of the Czech Republic

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## **1. INTRODUCTION**

Understanding the causes of biological invasions is a key topic of ecological research in the last decades (Sol et al., 2012). The reason is obvious - invasive plants affect natural communities, displacing native species and changing vegetation structure, causing reduction in diversity in the affected areas (Hejda et al., 2009; Powell et al., 2013), undermine the functioning of the whole ecosystems (Richardson and Pysek, 2012) and cause significant economic losses (Zavaleta, 2000). It is therefore in the interest of our society to determine the causes of species invasiveness and to try to prevent the emergence of new invasive species.

As there are many species which are exotic to the specific area but not necessarily invasive it is important to determine the mechanisms distinguishing between these two groups of species. If the species reaches new area it has to overcome certain barriers and there is possibility to define the degree of invasiveness of the species according to which barriers it has overtaken (Richardson et al., 2000). First barrier which species has to deal with is geographical – this part is called colonization and is often caused by humans. After that, species tries to build self-perpetuating colony. This phase is called naturalization. The last stage is when the species disperses within the resident flora and becomes invasive.

### **1.1. Success of invasive plants**

Many previous studies explored the properties that potentially cause invasiveness of plant species and thus differentiate invasive species from their non-invasive relatives. One possible property causing invasiveness is high phenotypic plasticity (Daehler, 2003). High phenotypic plasticity helps plants to adapt to different conditions (Schlichting, 1986) thus they can spread better and become invasive (Porté et al., 2011; Skalova et al., 2012). Another possible mechanism is higher ability to use resources (Dick et al., 2014) which is connected with higher ability to compete to other species (Doyle et al., 2003; Mal et al., 1997). Invasive plants are also often connected with high generative reproduction and high ability to disperse seeds (Burns et al., 2013; Moravcova et al., 2010). Invasive species can produce more seeds which are easily dispersible by wind or human and often delay germination to the spring of the next year waiting for convenient conditions. Another trait which allow species to become invasive is high growth rate during the early stages of the life-cycle (Burns, 2004; Leishman

et al., 2014). Thanks to this, species are bigger than others and can better use resources as for example sunlight. Plant invasiveness is also connected to small genome size (Kubesova et al., 2010). Species with small genome produce many light seeds with fast establishment (Rejmanek and Richardson, 1996) and thus have an advantage in temporal habitats as invasive species often occur in disturbed habitats (Bennett, 1987). While all these traits show some differences between invasive and non-invasive species, there is still a lot of variation that remains to be explained (Rejmanek and Richardson, 1996; van Kleunen et al., 2010).

Another possible explanation for the success of invasive plant species, which has been proposed recently, is the feedback between the plants and soil in which they grow (Bever, 1994; Callaway et al., 2004; Kulmatiski et al., 2008). This plant-soil feedback is a relationship in which the plant affects the composition of the soil and such modified soil affects growth of the plants (Bever et al., 1997). Commonly, two types of feedback, intraspecific and interspecific (sometimes referred to as direct and indirect), are distinguished. Intraspecific feedback expresses the influence of the species on performance of the same species. Interspecific feedback describes the effect of one species on performance of other species via soil (Bever et al., 1997). Plant-soil feedback can be positive or negative (Bever, 1994). Positive feedback leads to support of further growth of plant species, negative feedback leads to its suppression.

Both biotic and abiotic components of soil are involved in plant-soil feedback. Abiotic feedback is mainly about depletion of nutrients, discharge of various exudates into the soil by the plant and change of soil pH. This type of feedback is usually less species specific (Aerts and Chapin, 2000). In contrast, plant-soil feedback controlled by biotic components of soil (i.e. soil organisms) can be highly species specific (Van Der Putten, 2003). Abiotic and biotic plant-soil feedbacks do not affect plant separately – the resulting feedback is composed of these two effects.

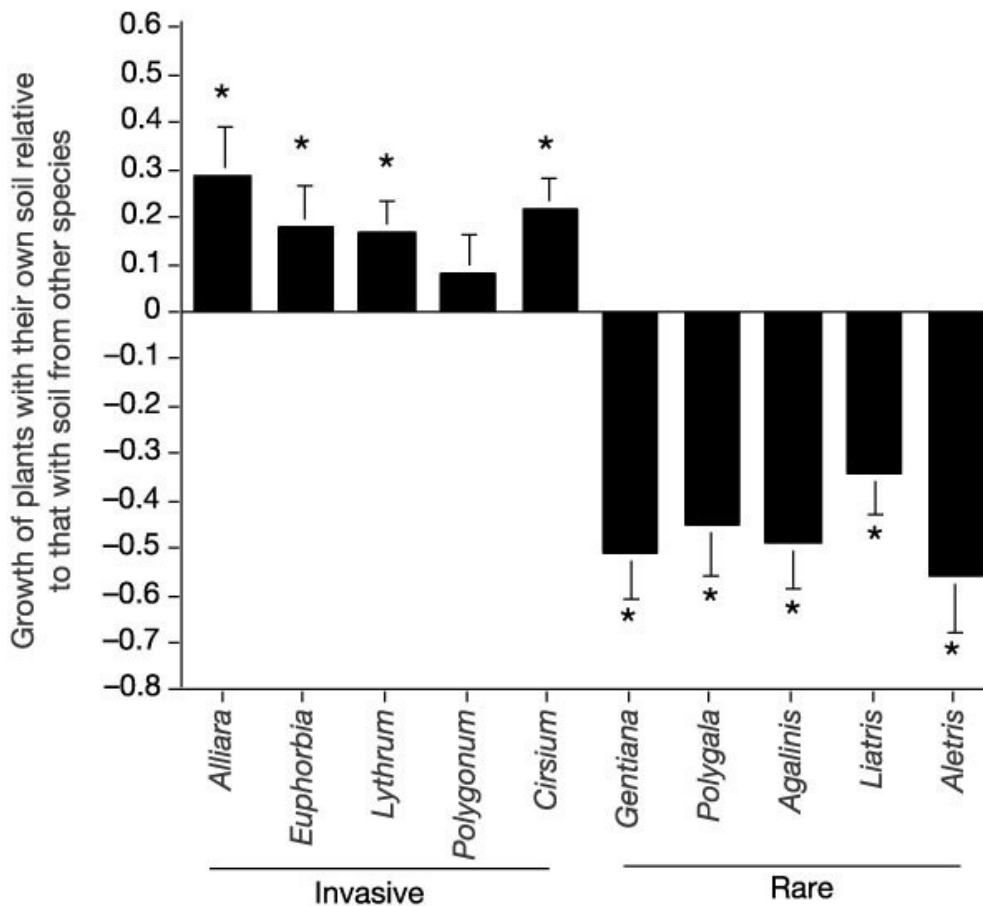
## **1.2. Intraspecific plant-soil feedback and invasive plants**

The importance of interspecific plant soil feedback for plant invasiveness which may be given by negative impact of invasive plants on soil environment, either through production of allelopathic substances secreted into the environment, or depletion of nutrients necessary for the growth of native plants has been known for quite some time (Del Fabbro and Prati, 2015;



Wardle et al., 1998). Similar effects caused by changes in composition of soil communities have been described relatively recently (Mangla et al., 2008; Shannon et al., 2014; van der Putten et al., 2013; Yang et al., 2014).

In addition to interspecific feedback, plant invasiveness may be also linked to the intensity of intraspecific feedback. Kulmatiski et al. (2008) demonstrated that majority of plants show negative intraspecific feedback which arises either via depletion of necessary nutrients from the soil, or the accumulation of species specific pathogens. It is, however, expected that dominant species can alter the composition of the soil in their favour and thus show positive intraspecific plant-soil feedback (Anacker et al., 2014; Klironomos, 2002) (Fig. 1). As the invasive species are commonly being dominant in their new environment (Hobbs et al., 2006), it can be expected that intraspecific positive plant-soil feedback could be an important factor allowing the invasive species to achieve their dominant position and become invasive.



**Fig. 1:** Dominant (invasive) species have higher growth in comparison with rare species (non-invasive) if growing in their own soil. It means that dominant species experience intraspecific plant-soil feedback which is positive and allow them to become dominant. In contrast rare species experience negative intraspecific plant-soil feedback. (Klironomos, 2002)

Existence of positive intraspecific feedback in invasive species can be also expected based on the Enemy Release Hypothesis (Elton, 1958). When the invasive plants are moving to the secondary range, they escape their natural, species-specific, pathogens and these thus cannot be accumulated in the soil and cannot negatively affect plant growth (Colautti et al., 2004; Levine et al., 2006). While generalists can attack both the original and introduced species, their influence on the regulation of population is usually considerably smaller than that of specialists (Colautti et al., 2004). In addition, soil symbionts, such as AM fungi, whose accumulation in the soil leads to positive intraspecific feedback between the plant and the soil have usually lower host specificity than pathogens (Smith, 2008) and the invasive plants can thus profit from their accumulation.

Example of positive intraspecific plant-soil feedback via enemy release is invasion of *Centaurea maculosa* (Callaway et al., 2004). *C. maculosa* is native in Europe, where it

cultivates soil biota with negative effects on its growth. On the other hand, in invasive range in North America, *C. maculosa* change the composition of soil in their favor, probably because lack of specific pathogens from Europe. Another study (van Grunsven et al., 2007) comparing three exotic and three native species demonstrated that exotic species experienced less negative intraspecific plant-soil feedback than natives due to enemy-release.

Some plants are also able to influence nutrient cycling and thus become invasive. *Bromus tectorum*, which is invasive in the western USA, is able to release twice as much N from its roots as non-invasive *Agropyron cristatum* although it has half the root abundance and lower root N concentrations (Morris et al., 2016). *B. tectorum* is probably able to build faster cycling and mineralizing environment in soil compared to other species (Stark and Norton, 2015). Another possible mechanism of positive intraspecific plant-soil feedback in invasive species is influencing of microbial community which results in higher or lower occurrence of this biota. *Impatiens glandulifera* alters the abundance of arbuscular mycorrhizal fungi - in self-conditioned soil is this abundance lower and it leads to higher plant biomass of *I. glandulifera* (Pattison et al., 2016). It may seem strange as relationship between plants and AMF is usually considered as more or less positive but it can also switch into parasitism (Gange and Ayres, 1999). High abundance of AMF in unconditioned soil thus has negative effect on plant growth as AMF take too much carbon from plant and plant uptake of P from fungi does not compensate for these losses. Positive intraspecific plant-soil feedback was also found in invasive species *Lespedeza cuneata* (Coykendall and Houseman, 2014) and *Heracleum mantegazzianum* (Dostal et al., 2013). Although *H. mantegazzianum* alters the soil in their favor, after some time this positive feedback turns into negative and it leads to coexistence with other species.

Also, other studies showing positive intraspecific plant-soil feedback in invasive species which can leads to their invasiveness (Gundale et al., 2014; Maron et al., 2014; Yang et al., 2013). Positive intraspecific plant-soil feedback was also detected in a recent review (Meisner et al., 2014), which additionally demonstrated that the effects strongly differ also between different life-forms. Importance of positive intraspecific plant-soil feedback for plant invasiveness has also been supported by a modelling study of Levine et al. (2006).

All these studies, however, involved only one or few species or are purely theoretical. To understand the generality of these patterns, we, however, need studies on larger number of species, preferably at the same time and environment (Reinhart and Callaway, 2006). Very useful design to obtain such information is the comparison of species that are invasive in a

given territory, with species that are introduced but not invasive (Kubesova et al., 2010; Moravcova et al., 2010; van Kleunen et al., 2010)).

Although it might seem from the cited literature that we already have achieved considerable knowledge about the processes driving plant invasions, the opposite is in fact true. The research that has been done so far provided only snapshots of ecological processes in invasion mainly because it was focused on a single or only few focal species or only few species traits. Although this brings precise view on plant-soil feedback and its influence on single plant species, it is necessary to move on and significantly improve our understanding of invasive mechanisms since invasions are causing truly enormous socio-economic troubles. To achieve it, it is necessary to move from rather simple experiments to more complex studies that will provide us with more power to generalize and also application for further fight with invasive plant species (van Kleunen et al., 2014).

Therefore, I will conduct an experiment on highly replicated introduced species and explore the importance of intraspecific plant-soil feedback as a potential mechanism driving species invasion.

### **1.3. Aims**

The aim of my diploma thesis is to test the hypothesis that intraspecific plant-soil feedback can be used as an explanation of plant invasiveness. I will examine the effect of intraspecific plant-soil feedback on 34 species – these species are neophytes of the Czech Republic. Neophytes are plants which were introduced to the specific area after the discovery of America, approx. 1500 A.D. (Pyšek et al., 2002). Plants introduced before that date are archeophytes. Set of plants will include both invasive and non-invasive, but naturalized, species. I hypothesize that invasive species will affect soil in their favor, while non-invasive species will show negative intraspecific feedback.

I will examine intraspecific plant-soil feedback on different levels. First I will examine intraspecific plant-soil feedback for each species separately. I will examine if positive feedback with soil occurs more for invasive or non-invasive species or there is no trend. At the end I will also compare the relative importance of intraspecific plant-soil feedback for plant invasiveness with other plant traits.

Specifically, I ask the following questions:

1. What is the direction and intensity of intraspecific plant-soil feedback in neophytes of the Czech Republic?
2. Is there a significant difference in plant-soil feedback between invasive and non-invasive, but naturalized, plant species?
3. Is intraspecific plant-soil feedback better explanation of plant invasiveness than other plants characteristics studied previously (e.g. the size of the genome or reproductive traits)?

## 2. MATERIALS AND METHODS

### 2.1. Selection of plants and seed collection

For realization of my project I used the same set of species, which was used in studies of Kubesova et al. (2010) and Moravcova et al. (2010). I updated the status of individual plant species using Pysek et al. (2012). Some species were reclassified as invasive (originally naturalized), naturalized (originally invasive) and archeophytes (originally neophytes). From this set, I chose 34 neophytes for which I was able to collect seeds and planted them (Appendix 1). The plant set included 17 invasive species and 17 naturalized, but non-invasive species. *Rudbeckia hirta* had status casual but it was merged with naturalized non-invasive species.

There are 28 genera which belong to 15 families in the species set. The most frequent family is *Asteraceae* (11 species). Invasive and non-invasive species contain approximately the same proportion of annuals (10 invasive, 8 non-invasive) and perennials (7 invasive, 9 non-invasive). There were three life-history categories – annual, biennial and perennial. But as biennials were under represented (only two species) this category was merged with category annuals.

Using this species list has several advantages. First, there already is the information on the localities at which seeds of these species can be easily collected (provided by L. Moravcová). Second, there is good information on germination requirements of the species (provided by L. Moravcová). Third, there is a lot of previous information on various traits of these species (Kubesova et al., 2010; Moravcova et al., 2010) allowing to determine the relative importance of the intensity of plant-soil feedback compared to other plant traits in invasiveness of plants. Information on other traits is also available elsewhere in the literature.

Seeds were collected during growing season of 2015 and 2016. To account for possible differences between populations, I collected seeds from different localities (min. 1, max. 3) from at least ten different individuals at each locality. Seeds from each population (locality) were used separately in the experiment. Seeds were stored in paper sacks at room temperature. If it was not possible to collect the seeds within localities, the seeds were ordered from Planta Naturalis company (Appendix 1).

I put species that needed stratification on filter paper into Petri dishes and stored them in the fridge for eight weeks before sowing. Stratified seeds were regularly watered with

distilled water. If seeds got moldy, I washed them in a solution of Previcur Energy (Bayer Garden) and water (1:100). All seeds were washed in a solution of Savo and water (1:10) before sowing and thus sterilized.

## **2.2. Experiment**

For realization of the project, I used the method of two-phase experiment (Bever, 1994; Brinkman et al., 2010). The first phase is called soil conditioning – influencing of soil by the plant. In the second (feedback) phase, a target plant is grown in the conditioned soil.

Because the total number of pots was high, not all plant species were planted simultaneously. The experiment was divided into two years (2015, 2016); about half of plant species (both invasive and non-invasive) was cultivated each year. Soil used in the experiment was prepared during the first year (in May 2015) by mixing common garden soil and sand in the ratio 1:1. Prepared and mixed soil was left in the garden covered with a tarpaulin. Soil was mixed before each use.

### **2.2.1. Conditioning phase**

The aim of the first phase was to prepare soil conditioned by each plant species. I used twenty pots (10 x 10 x 10 cm) with soil for each plant species and populations – ten pots for conditioned soil and ten pots for control soil (Fig. 2). I sowed ten seeds of each population of the plant species to ten pots (conditioned soil) and kept other ten pots vacant – without seeds (control soil). In some cases, I used twenty seeds instead of ten because these seeds were too small or light and I wanted to be sure that at least some of the seeds will germinate. Specifically, 20 seeds were used for *Conyza canadensis*, *Digitalis purpurea*, *Galinsoga parviflora*, *Oenothera glazioviana*, *Sisymbrium loeseli* and *Solidago canadensis*. Seeds were sown approximately one centimeter under the surface.

Pots were stored in identical conditions in the garden and regularly watered (two times per day). After germination, seedlings were counted and removed from pots except for one seedling to avoid strong density dependence effects between the plants but to ensure that the soil is thoroughly cultivated. Removed plants were placed in paper bags (roots were washed with water), dried (60°C, 48 hours) and weighed (above and below-ground biomass

separately). The remaining plant was left in the pot for 12 weeks. During this time, I removed all weeds from pots to ensure that the soil was not conditioned by another plant species. After 12 weeks, plants were harvested, placed in paper bags (roots were additionally washed to remove residual soil), dried (60°C, 48 hours) and weighed (above and below-ground biomass separately). When harvesting plants I poured content of each pot on a tray and picked up all above and below-ground biomass – there was almost nothing left apart of few very small root hairs. The soil from each pot was then returned to that pot. Conditioned and control soil was used in the second phase of the experiment. Conditioning phase took place from April to June (both years).

I took ten leaves of each species to gain specific leaf area (SLA), because this trait is not known for all my species. I cut the leaves from mature plant (closely before harvesting) without leafstalk, pressed and dried them and then weighed and scan to the computer. To gain a leaf area I used program ImageJ. Then I calculated SLA for each species as mean of areas of each leaf divided by its weight.



**Fig. 2:** Photo of pots with growing plants during the first phase. Plants influence the soil for the second phase. Experimental garden in Průhonice, **Botanický ústav AV ČR, v. v. i.**



### **2.2.2. Feedback phase**

In the second phase of the experiment, seeds of all species were sown in the two types of soil: i) conditioned soil, ii) control soil. The number of seeds in each pot was ten due to possibility to observe germination in conditioned and control soil. In some cases I used twenty seeds (see 2.2.1. Conditioning phase). Seeds were sown approximately one centimeter under the surface.

After germination, seedlings were counted and removed from pots except for one seedling. Removed plants were placed in paper bags, dried and weighed using the same methods like in the first phase of the experiment. The remaining plant was left in the pot for 12 weeks. During this time I removed all weeds from pots to ensure that the results will not be influenced by other species. Pots were kept in identical conditions in the garden and regularly watered (two times per day). After 12 weeks, the plants were harvested, dried and weighed using the same methods like in the first phase of the experiment. Because some species started to flower and produce seeds at the end of the second phase I picked up all seeds from plants, counted and weighed them. Feedback phase took place from July to September (both years).

### **2.3. Data processing and statistical analyses**

#### **2.3.1. Measured variables**

In my experiment I recorded the number of plants in pots during the second phase so I could examine seed germination in conditioned and control soil. But I had data on germination only for 29 species (14 invasive and 15 non-invasive) as some samples were discarded. Specifically, number of plants in pots was not known for these species – *Abutilon theophrasti*, *Amaranthus albus*, *Ambrosia artemisiifolia*, *Cannabis ruderalis*, *Echinops sphaerocephalus*, *Erigeron annuus*.

Besides I measured plant biomass in each pot. There was possibility to use biomass of all plants which germinated in pots or biomass of one plant which was left in the pot for 12 weeks. Because these two variables were highly correlated (Pearson correlation coefficient, Table 1) I used only biomass of one plant remaining in pot for 12 weeks in all analyses. It is in coincidence with other studies on intraspecific plant-soil feedback (Birnbaum and Leishman, 2013; Klironomos, 2002). I measured aboveground and belowground biomass of plants separately. It means that I had these four variables – belowground biomass,

aboveground biomass, total biomass (above and belowground together), root-shoot ratio. In analyses I used only total biomass and root-shoot ratio as above and belowground biomass was closely correlated with total biomass (Pearson correlation coefficient, Table 1). All biomass used in analyses was from the second (feedback) phase. Biomass from the first (conditioning) phase was used only in comparison of biomass from the first and second phase (see 2.3.2. Difference between conditioned and control soil for individual species).

	Shoot	Root	Root-shoot ratio	Biomass of one plant	Total biomass
Shoot	1	0.27	-0.25	0.72	0.66
Root	0.27	1	0.40	0.84	0.74
Root-shoot ratio	-0.25	0.40	1	0.14	0.08
Biomass of one plant	0.72	0.84	0.14	1	0.9
Total biomass	0.66	0.74	0.08	0.9	1

**Table 1: Pearson correlation coefficients for variables of biomass measured in the experiment.** Correlations for all variables were significant. I used only biomass of one plant and root-shoot ratio in the analysis as other variables were highly correlated with biomass of one plant. Shoot – aboveground biomass, Root – belowground biomass, Root-shoot ratio – ratio of root and shoot, Biomass of one plant – biomass of plant remaining in pot for all 12 weeks, Total biomass – biomass of all plants germinated in pot.

Additionally, as some species started to flower in the second phase I also measured the number of the seeds and their weight. I had this data for 6 species (3 invasive and 3 non-invasive) – *Amaranthus albus*, *Ambrosia artemisiifolia*, *Bidens frondosa*, *Galinsoga parviflora*, *Chenopodium strictum*, *Xanthium albinum*.

### 2.3.2. Difference between conditioned and control soil for individual species

The first part of the statistical analyses was to determine whether there is a significant difference between plant biomass of the second phase in conditioned and control soil. I used ANOVA model to test it. As a dependent variable, I used total biomass or root-shoot ratio. An

explanatory variable was soil type (conditioned or control soil). The analysis was conducted for each species separately. Because some species originated from multiple localities I used mixed effect model instead of ANOVA. A dependent variable was total biomass or root-shoot ratio and an explanatory variable was soil type same as in ANOVA model. The locality was a random factor in the mixed effect model (only if the number of conditioned and control pots from each locality was higher than five).

Then I examined whether plant biomass from the second (feedback) phase is dependent on the biomass of the first (conditioning) phase. In these tests I used the biomass of plants from the second phase as a dependent variable and biomass from the first phase as an explanatory variable. I tested dependence using linear regression for each species separately.

The difference between seed germination of the second phase in conditioned and control soil was tested using ANOVA model where number of germinated seeds was dependent variable and type of soil was explanatory variable. The analyses were performed for each species separately; in case of multiple localities per species I used the same mixed effect model like in the previous analysis with number of germinated seeds as a dependent variable, soil type as an explanatory variable and locality as a random factor.

I also analyzed the difference between production of seeds in conditioned and control soil. There were two variables – number of seeds and their weight. I tested the difference using ANOVA model with number of seeds or weight of seeds as a dependent variable and soil type as an explanatory variable. The analyses were performed for each species separately; in case of multiple localities per species I used the same mixed effect model like in the previous analyses with number of seeds or weight of seeds as a dependent variable, soil type as an explanatory variable and locality as a random factor.

In case of mixed effect model I used the Likelihood Ratio Test to attain p-values. The null model was the model without an explanatory variable (soil type), my model was the model with an explanatory variable (soil type). I compared these two models (null model and my model) with each other to see the p-values.

The variables were transformed if it was necessary.

### 2.3.3. Index of intraspecific plant-soil feedback

To answer the main question, i.e. if there is a significant difference in PSF between invasive and non-invasive, but naturalized, plant species, I calculated feedback index based on seed germination for each species (Brinkman et al., 2010). I calculated it as

$$\ln(A/B),$$

where **A** was number of germinated seeds in conditioned soil and **B** was mean number of germinated seeds in control soil. I compared feedback index between invasive and non-invasive species using mixed effect model with locality and species as a random factor. Feedback index was used as a dependent variable and status of plant (invasive vs. non-invasive) was used as an explanatory variable.

I calculated also feedback index based on biomass of plants and number and weight of seeds produced by plants in the second phase (using the same method for calculating index as in germination) and I did the same type of analyses with these indexes as with feedback index based on germination.

I correlated indices based on seed germination, plant biomass and production of seeds (number and weight) (Table 2). As index calculated from number of seeds and index calculated from weight of seeds were highly positively correlated ( $r = 0.96$ ,  $p < 0.001$ ) I used only index based on number of seeds in the analyses.

	plant biomass	germination	number of seeds	weight of seeds	root-shoot ratio
plant biomass	1	0.07	<b>0.70</b>	<b>0.68</b>	-0.03
germination	0.07	1	0.11	0.19	<b>-0.18</b>
number of seeds	<b>0.70</b>	0.11	1	<b>0.96</b>	-0.01
weight of seeds	<b>0.68</b>	0.19	<b>0.96</b>	1	-0.04
root-shoot ratio	-0.03	<b>-0.18</b>	-0.01	-0.04	1

**Table 2: Pearson correlation coefficients for feedback indices.**

Feedback index was counted for each pot, locality and plant separately as  $\ln(\text{variable in conditioned soil} / \text{mean variable in control soil})$ . The variables: plant biomass, germination of seeds, number and weight of seeds from the second phase, root-shoot ratio. Significant correlations are in bold. I did not use the weight of seeds in analyses as it was highly correlated with the number of seeds.

#### **2.3.4. Intraspecific plant-soil feedback and other traits**

To test whether intraspecific plant-soil feedback is better explanation of plant invasiveness than other plants characteristics studied previously I chose a set of plants traits which are usually connected with invasiveness (Table 3). These included data on generative reproduction, dispersal and genome size, time of first introduction, life history, plant height and start and end of flowering. There are also feedback indices based on germination and plant biomass and SLA – these traits were measured within the experiment. The data on time of first introduction was also used to test the hypothesis that the intensity of plant-soil feedback is significantly affected by residence time. If some of the data will be missing for some of the species, these traits will be tested in partial datasets.

I counted correlations among all traits (Pearson correlation coefficient, Appendix 2). As all correlations of traits showed low dependence I used all of them in my analyses.

I tested each plant trait separately using *glm* model (binomial distribution). Status of plant (invasive vs. non-invasive) was used as a dependent variable and individual traits were used as an explanatory variable. To find what is the strength of trait in comparison with others I deducted AIC of model with trait from AIC of null model and then compared these numbers.

All analyses were done in R (version 3.2.5).

<b>plant trait</b>	<b>source</b>
Feedback index based on germination	own data
Feedback index based on biomass	own data
SLA (g/cm <sup>2</sup> )	own data
Life history (annual + biennial; perennial)	Pyšek et al. (2012)
First reported occurrence in the wild in the Czech Republic	Pyšek et al. (2012)
Releasing height of plant (m)	LEDA database
Type of dispersal	LEDA database
Start of flowering	Kubát et al. (2002)
End of flowering	Kubát et al. (2002)
Genome size	Kubesova et al. (2010)
Level of ploidy	Kubesova et al. (2010)
Number of propagules per plant	Moravcova et al. (2010)
Propagule weight (g)	Moravcova et al. (2010)
Propagule size (length and width, mm)	Moravcova et al. (2010)
Germination	Moravcova et al. (2010)
Seedling relative growth rate (RGR, g·g <sup>-1</sup> ·day <sup>-1</sup> )	Moravcova et al. (2010)
Seedling establishment	Moravcova et al. (2010)

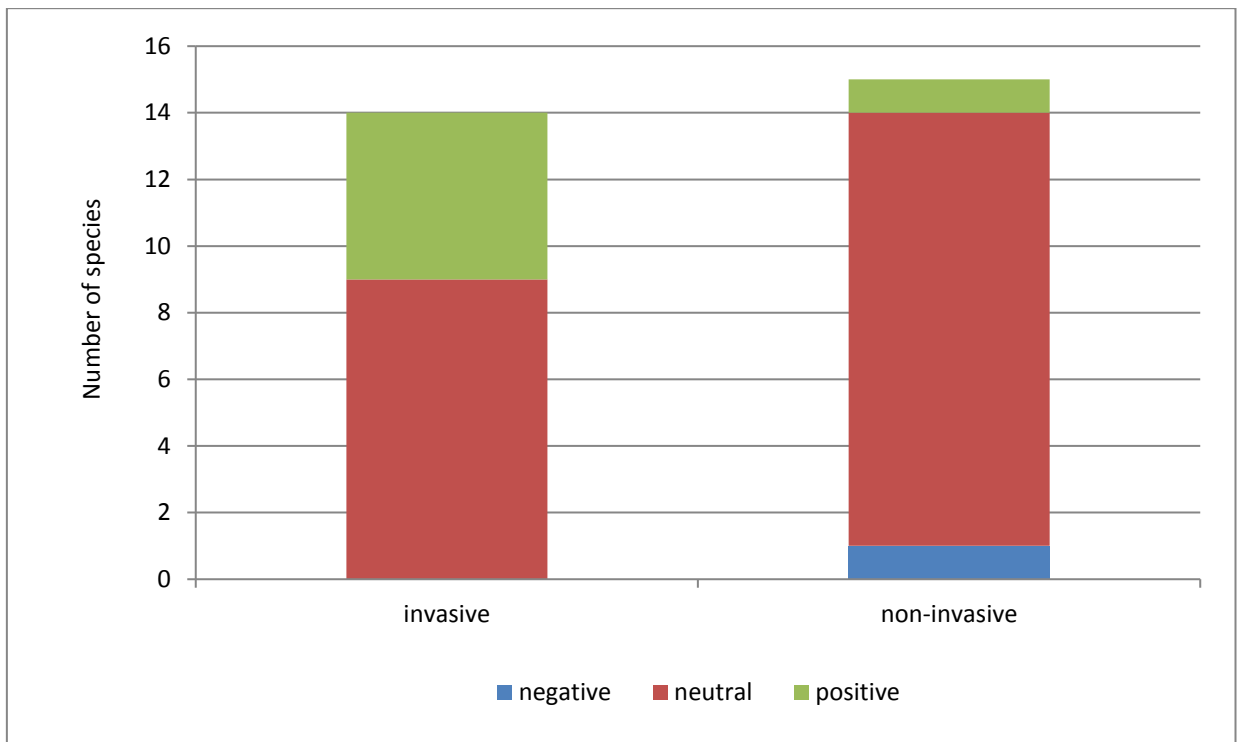
**Table 3: Plants traits used in analyses.**

The data on time of first introduction was also used although it is not a plant trait. For detailed description of individual traits see Appendix 3. The sources of data for each trait are written in column “source”.

### 3. RESULTS

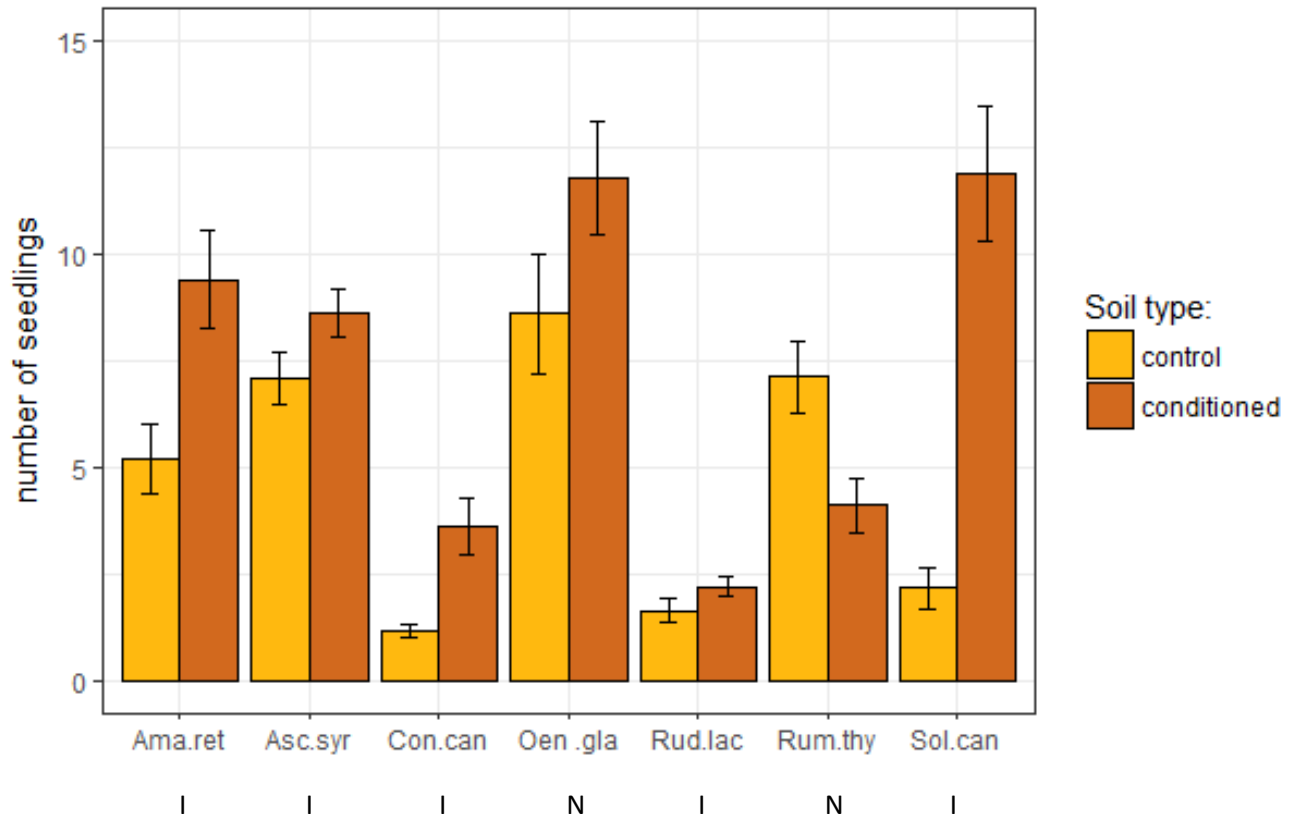
#### 3.1. Direction and intensity of intraspecific plant-soil feedback for each species

Significant difference between germination of seeds in control and conditioned soil occurred in 7 species ( $p \leq 0.05$ ). Six species germinated significantly better in conditioned soil – *Amaranthus retroflexus* (invasive), *Asclepias syriaca* (invasive), *Conyza canadensis* (invasive), *Oenothera glazioviana* (non-invasive), *Rudbeckia laciniata* (invasive) and *Solidago canadensis* (invasive) (Fig. 4). One species (*Rumex thyrsiflorus* – non-invasive) germinated significantly better in control soil. Twenty two species had no significant difference between germination of seeds in conditioned and control soil (Table 4) (Fig. 3).



**Fig. 3: Difference between germination of seeds in control and conditioned**

Five invasive species germinated significantly better in conditioned soil (positive), for nine invasive species the tests were not significant (neutral). One non-invasive species germinated significantly better in conditioned soil (positive), one non-invasive species germinated significantly worse in conditioned soil (negative), for thirteen species the tests were not significant (neutral).



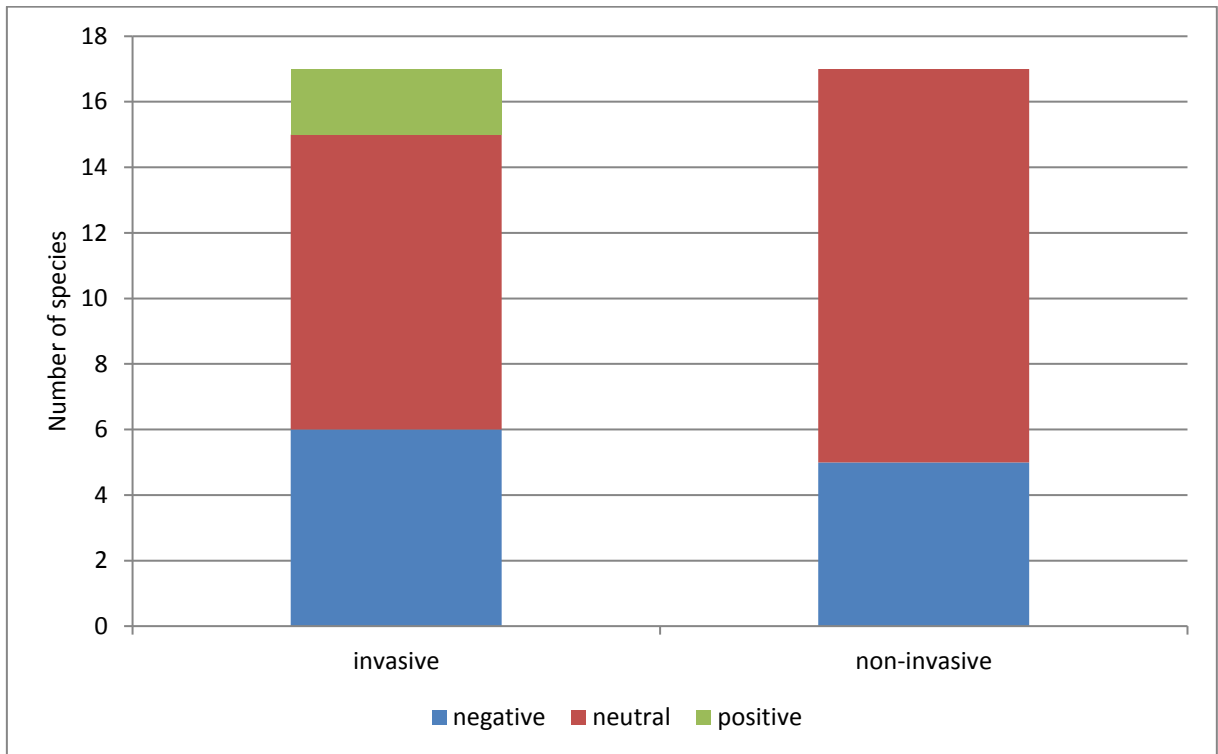
**Fig. 4: Significant differences between germination of seeds in control and conditioned soil**  
 Columns show mean and error bars show +/- standard error. Only species with significant differences in germination between conditioned and control soil are shown. Shortcuts of species names are written in Appendix 1. The letter under species name is its status (I – invasive, N – non-invasive).



species	status	germination	biomass	R:S ratio	seed (n.)	seed (w.)
<i>Abutilon theophrasti</i>	N	x	<b>0.0058</b>	<b>0.0447</b>	x	x
<i>Amaranthus albus</i>	N	x	<b>0.0055</b>	<b>0.0047</b>	<b>0.0020</b>	<b>0.0177</b>
<i>Amaranthus powellii</i>	I	0.1794	<b>0.0246</b>	0.9587	x	x
<i>Amaranthus retroflexus</i>	I	<b>0.0002</b>	<b>0.0112</b>	0.9100	x	x
<i>Ambrosia artemisiifolia</i>	I	x	0.3906	0.9596	0.8621	0.5679
<i>Asclepias syriaca</i>	I	<b>0.0453</b>	<b>0.0134</b>	<b>0.0038</b>	x	x
<i>Bidens frondosa</i>	I	0.8381	0.0790	<b>0.0042</b>	0.2159	0.4003
<i>Cannabis ruderalis</i>	I	x	0.4985	0.9859	x	x
<i>Conyza canadensis</i>	I	<b>0.0215</b>	<b>0.0005</b>	<b>0.0007</b>	x	x
<i>Datura stramonium</i>	N	0.9415	0.8114	0.9222	x	x
<i>Digitalis purpurea</i>	N	0.2647	0.2593	<b>0.0033</b>	x	x
<i>Echinops sphaerocephalus</i>	I	0.2301	0.2380	0.0583	x	x
<i>Erigeron annuus</i>	I	x	0.3137	0.0975	x	x
<i>Galinsoga parviflora</i>	I	0.7505	0.8859	<b>0.0145</b>	0.6385	0.4364
<i>Geranium pyrenaicum</i>	N	0.0799	0.0593	<b>0.0009</b>	x	x
<i>Hesperis matronalis</i>	N	0.3931	0.1327	<b>0.0496</b>	x	x
<i>Chenopodium strictum</i>	N	0.1428	<b>&lt; 0.001</b>	0.4524	<b>0.0011</b>	<b>0.0009</b>
<i>Impatiens parviflora</i>	I	0.8020	<b>0.0295</b>	0.9221	x	x
<i>Lupinus polyphyllus</i>	I	0.5045	0.9487	0.2195	x	x
<i>Lychnis coronaria</i>	N	0.1576	<b>0.0019</b>	<b>0.0069</b>	x	x
<i>Medicago sativa</i>	N	0.0871	<b>0.0065</b>	0.4959	x	x
<i>Mimulus guttatus</i>	N	0.6437	0.3796	0.5129	x	x
<i>Oenothera biennis</i>	N	0.1038	0.3595	<b>0.0402</b>	x	x
<i>Oenothera glazioviana</i>	N	<b>0.0445</b>	0.0833	0.9883	x	x
<i>Rudbeckia hirta</i>	N	0.0596	0.8072	0.9326	x	x
<i>Rudbeckia laciniata</i>	I	<b>0.0257</b>	0.3715	0.1550	x	x
<i>Rumex alpinus</i>	I	0.8846	<b>&lt; 0.001</b>	0.0611	x	x
<i>Rumex thyrsoiflorus</i>	N	<b>0.0033</b>	0.0923	0.0672	x	x
<i>Sisymbrium altissimum</i>	N	0.3143	0.1408	0.2003	x	x
<i>Sisymbrium loeselii</i>	I	0.0868	<b>0.0189</b>	<b>0.0388</b>	x	x
<i>Solidago canadensis</i>	I	<b>4.9E-05</b>	<b>0.0386</b>	0.1634	x	x
<i>Telekia speciosa</i>	I	0.6193	0.4294	0.6478	x	x
<i>Trifolium hybridum</i>	N	0.1747	0.7174	0.5585	x	x
<i>Xanthium albinum</i>	N	0.6912	0.7230	0.5278	0.0550	0.2011

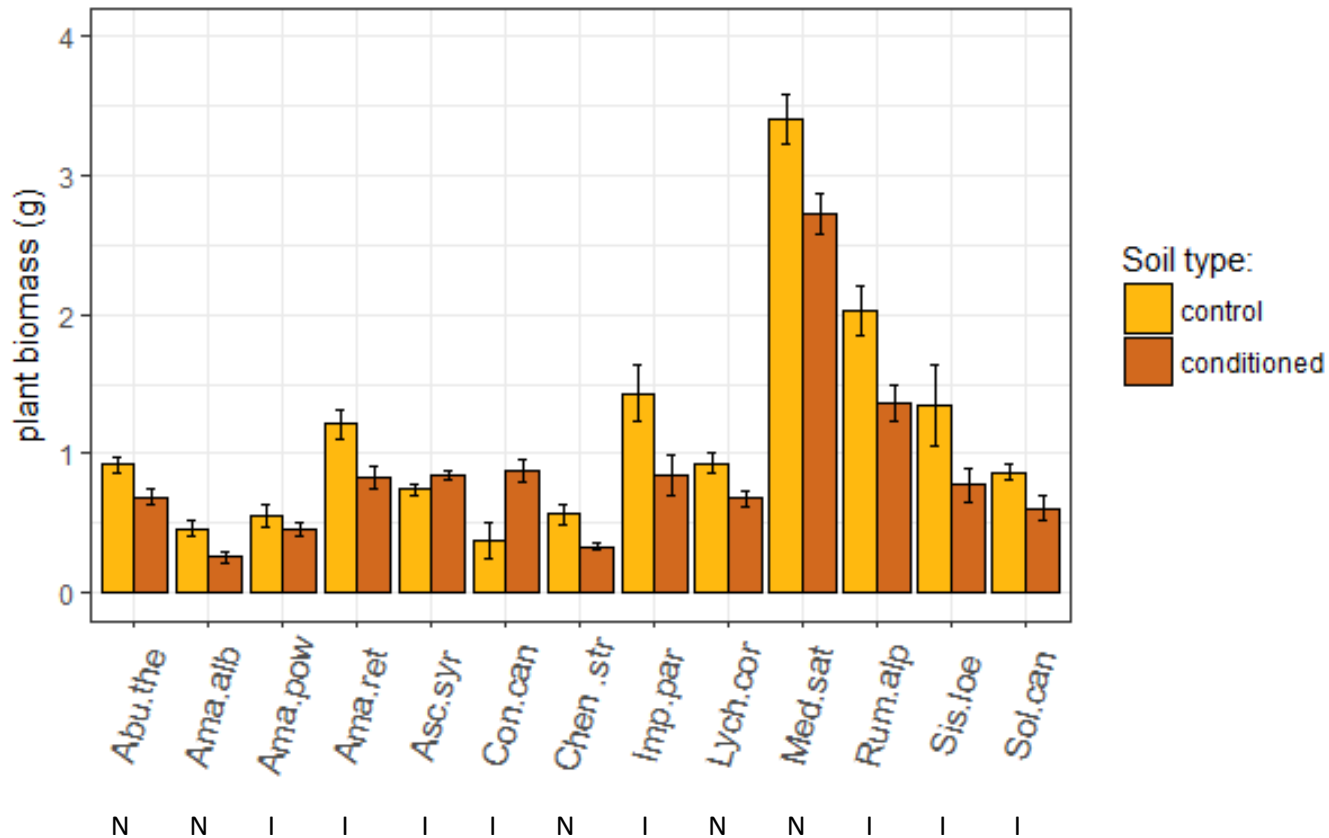
**Table 4:** Five variables were tested for difference between conditioned and control soil for each species. Used analyses were ANOVA or in case of multiple localities mixed effect model with locality as a random effect. Numbers are p-values – these in bold and color are significant ( $p \leq 0.05$ ), these in bold but no color are almost significant. **Green color** – the variable was higher in conditioned soil, **blue color** – the variable was higher in control soil. x – there were no data for the species. Variables - **germination:** number of seeds germinated in pot, **biomass:** biomass of plant growing in pot, **R:S ratio:** root-shoot ratio of plant, **seed (n.):** number of seeds produced by plant, **seed (w.):** weight of seeds produced by plant. **Status** means if plant is invasive (I) or non-invasive (N).

Significant difference between plant biomass in control and conditioned soil occurred in 13 species ( $p \leq 0.05$ ). Two species had higher biomass in conditioned soil – *Asclepias syriaca* (invasive) and *Conyza canadensis* (invasive) (Fig. 6). The other eleven species had higher plant biomass in control soil. Twenty one species had no significant difference between plant biomass in control and conditioned soil (Table 4) (Fig. 5).



**Fig. 5: Difference between biomass of plants in control and conditioned**

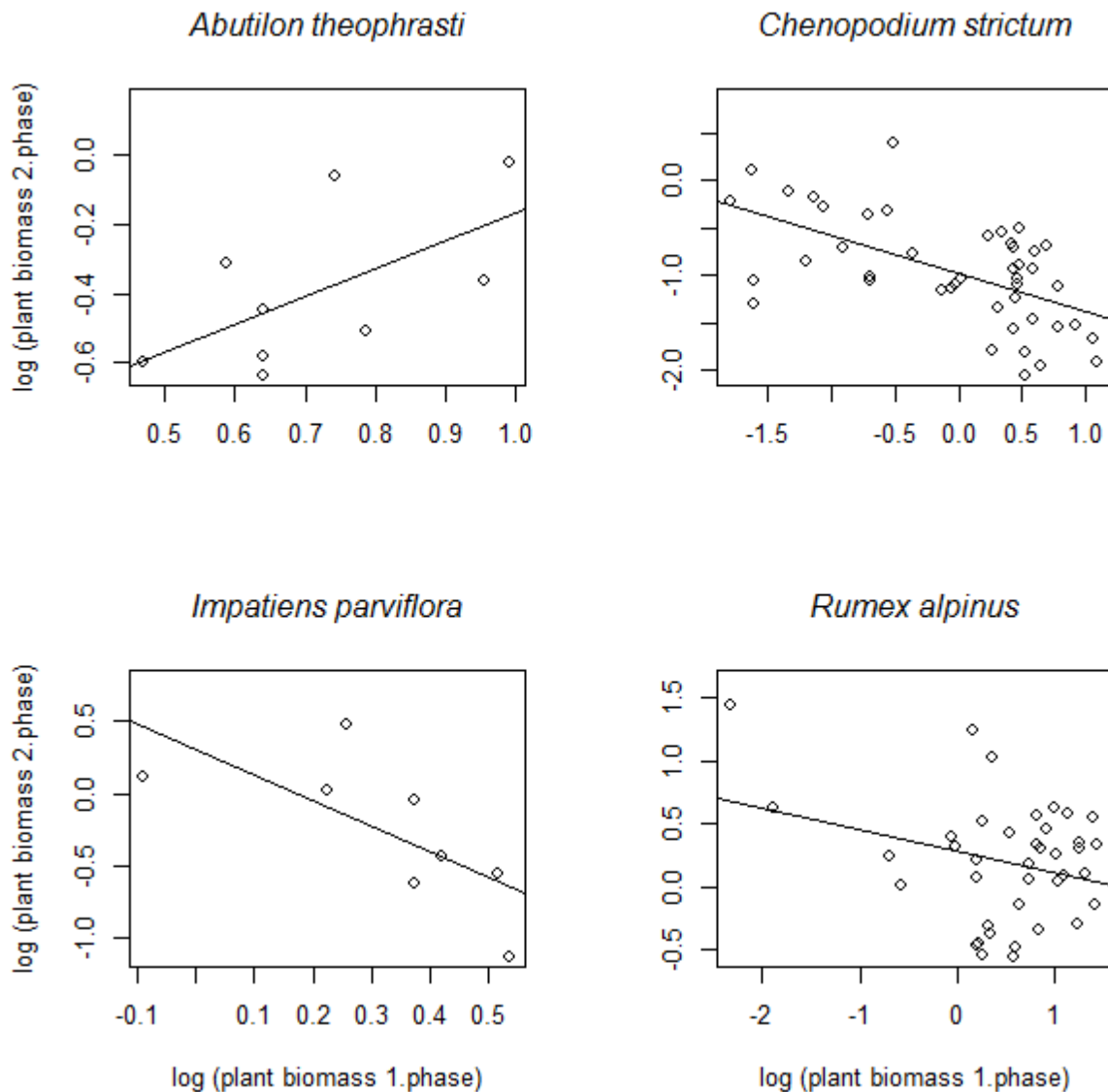
Two invasive species grew significantly better in conditioned soil (positive), six invasive species grew significantly worse in conditioned soil (negative), for nine species the tests were not significant (neutral). Five non-invasive species grew significantly worse in conditioned soil (negative), for twelve species the tests were not significant (neutral).



**Fig. 6: Significant differences between plant biomass in control and conditioned soil**

Columns show mean and error bars show +/- standard error. There are only species which significantly differ between plant biomass in conditioned and control soil. Shortcuts of species names are written in Appendix 1. The letter under species name is its status (I – invasive, N – non-invasive).

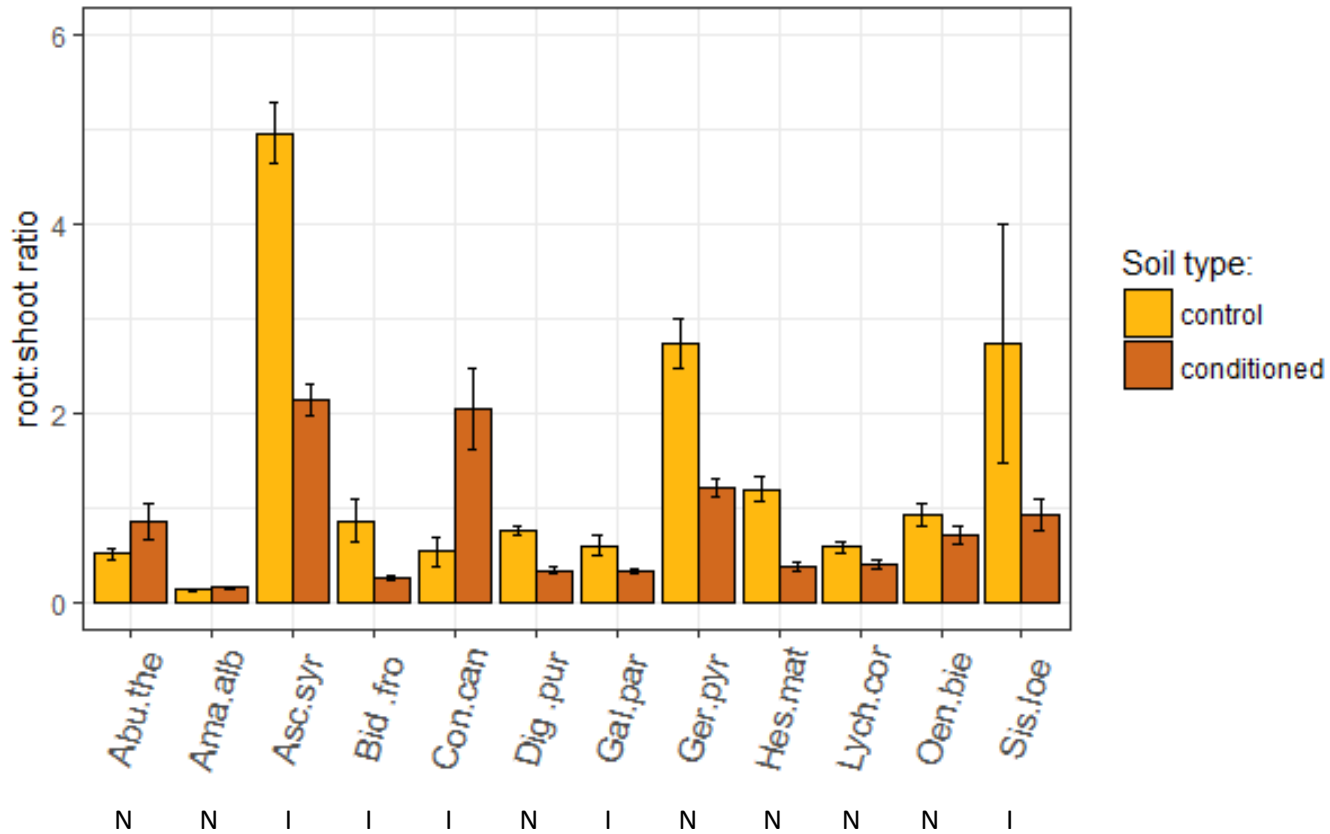
For species, which showed significant difference between biomass in conditioned and control soil, the dependence of plant biomass of the second phase on biomass of the first phase was tested. Marginally significant results occurred for four species – *Abutilon theophrasti* (non-invasive), *Chenopodium strictum* (non-invasive), *Impatiens parviflora* (invasive) and *Rumex alpinus* (invasive) (Fig. 7). *Abutilon theophrasti* showed marginally significant positive relationship between biomass of the second and first phase ( $p = 0.0848$ ). *Chenopodium strictum* ( $p = 0.079$ ), *Impatiens parviflora* ( $p = 0.0555$ ) and *Rumex alpinus* ( $p = 0.0724$ ) showed marginally significant negative relationship between biomass of the second and first phase.



**Fig. 7: Dependence of plant biomass of the second phase on plant biomass of the first phase**  
*A. theophrasti* showed positive relationship ( $p = 0.0848$ ). *Ch. strictum* ( $p = 0.079$ ), *I. parviflora* ( $p = 0.0555$ ) and *R. alpinus* ( $p = 0.0724$ ) showed negative relationship.

Significant difference between root-shoot ratio in conditioned and control soil occurred in 12 species ( $p \leq 0.05$ ). Three species had higher root-shoot ratio in conditioned soil – *Abutilon theophrasti* (non-invasive), *Amaranthus albus* (non-invasive) and *Conyza canadensis* (invasive). Nine species had higher root-shoot ratio in control soil – *Asclepias syriaca* (invasive), *Bidens frondosa* (invasive), *Digitalis purpurea* (non-invasive), *Galinsoga parviflora* (invasive), *Geranium pyrenaicum* (non-invasive), *Hesperis matronalis* (non-invasive), *Lychnis coronaria* (non-invasive), *Oenothera biennis* (non-invasive), *Solidago*

*canadensis* (invasive) (Fig. 8). Twenty two species had no significant difference between root-shoot ratio in conditioned and control soil. (Table 4).



**Fig. 8: Significant differences between root-shoot in control and conditioned soil**

Columns show mean and error bars show +/- standard error. There are only species which significantly differ between root-shoot ratio in conditioned and control soil. Shortcuts of species names are written in Appendix 1. The letter under species name is its status (I – invasive, N – non-invasive).

Significant difference ( $p \leq 0.05$ ) between production of seeds (both variables – number of seeds and their weight) in conditioned and control soil occurred in two species – *Amaranthus albus* (non-invasive), *Chenopodium strictum* (non-invasive). Both species showed better seed production in control soil. Other four species had no significant difference between production of seeds in conditioned and control soil (Table 4).

### 3.2. Index of intraspecific plant-soil feedback

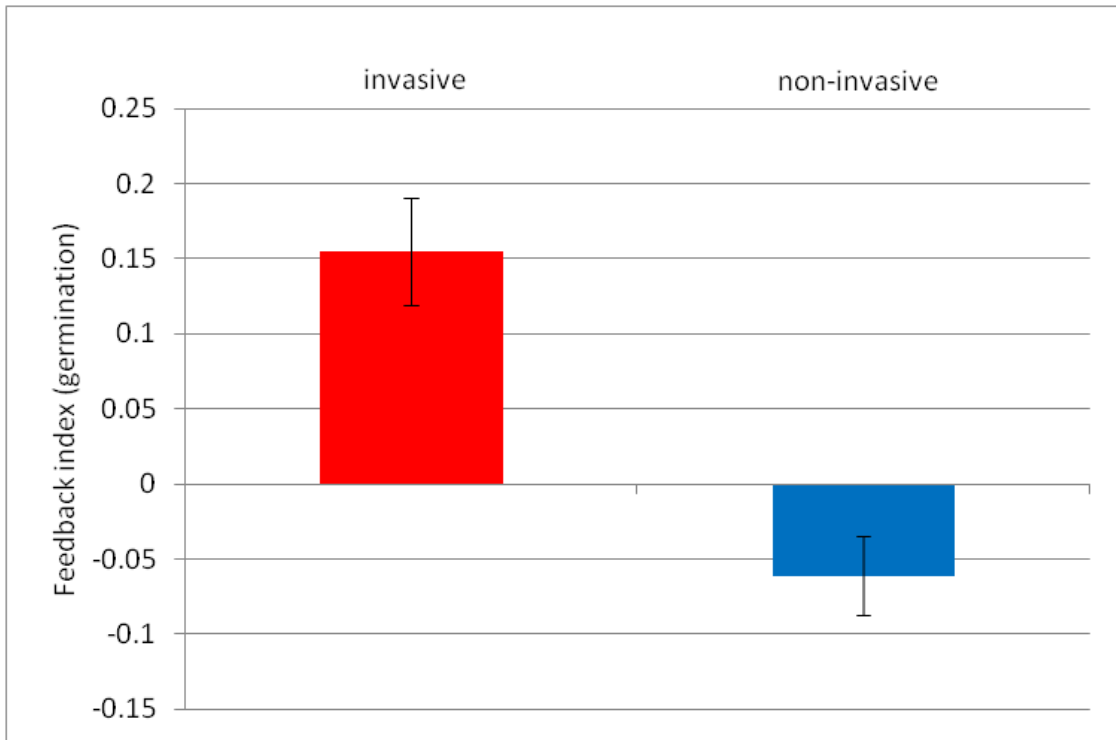
Feedback index based on seed germination varied significantly between invasive and non-invasive plant species ( $\chi^2(1)=5.153$ ,  $p=0.023$ ) (Table 5). Invasive plants had higher feedback index than non-invasive plants (see Fig. 9 and 10). It means that invasive plants germinated better in their own soil than non-invasive plants. Mean index of plant-soil feedback for each plant species is shown in Table 6.

	N	dev.	p-value
Feedback index (germination)	485	5.153	<b>0.023</b>
Feedback index (biomass)	569	2.883	0.090
Feedback index (root-shoot ratio)	567	0.607	0.436
Feedback index (number of seeds)	45	0.553	0.457

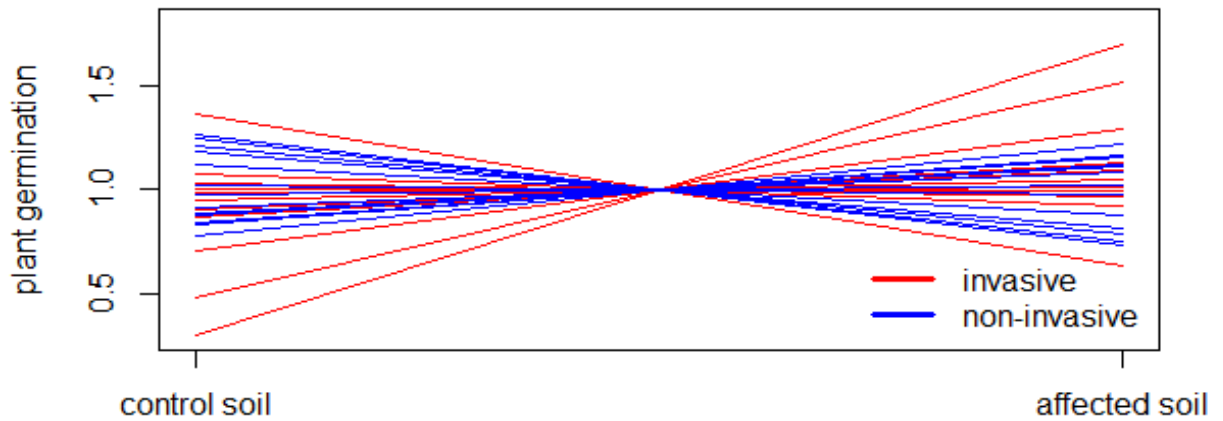
**Table 5:** Differences of feedback indices based on germination of seeds, plant biomass, root-shoot ratio and number of seeds between invasive and non-invasive plants. Used analysis was mixed effect model with locality and species as random factor. Significant p-value ( $\leq 0.05$ ) is in bold. Dev. indicates deviance explained by the given variable.

species	status	Feedback index (germination)	Feedback index (biomass)
<i>Abutilon theophrasti</i>	N	x	-0.314
<i>Amaranthus albus</i>	N	x	-0.676
<i>Amaranthus powellii</i>	I	0.080	-0.222
<i>Amaranthus retroflexus</i>	I	0.494	-0.431
<i>Ambrosia artemisiifolia</i>	I	x	0.142
<i>Asclepias syriaca</i>	I	0.235	0.036
<i>Bidens frondosa</i>	I	-0.102	0.164
<i>Cannabis ruderalis</i>	I	x	-0.055
<i>Conyza canadensis</i>	I	0.794	0.732
<i>Datura stramonium</i>	N	0.069	-0.141
<i>Digitalis purpurea</i>	N	0.420	-0.262
<i>Echinops sphaerocephalus</i>	I	-0.156	-0.171
<i>Erigeron annuus</i>	I	x	0.135
<i>Galinsoga parviflora</i>	I	-0.021	-0.339
<i>Geranium pyrenaicum</i>	N	0.325	-0.338
<i>Hesperis matronalis</i>	N	-0.409	-0.432
<i>Chenopodium strictum</i>	N	-0.351	-0.520
<i>Impatiens parviflora</i>	I	x	-0.631
<i>Lupinus polyphyllus</i>	I	-0.242	-0.079
<i>Lychnis coronaria</i>	N	-0.725	-0.426
<i>Medicago sativa</i>	N	0.179	-0.256
<i>Mimulus guttatus</i>	N	0.034	-0.131
<i>Oenothera biennis</i>	N	-0.487	-0.103
<i>Oenothera glazioviana</i>	N	0.208	-0.278
<i>Rudbeckia hirta</i>	N	0.346	-0.042
<i>Rudbeckia laciniata</i>	I	0.217	-0.291
<i>Rumex alpinus</i>	I	-0.052	-0.470
<i>Rumex thyrsoiflorus</i>	N	-0.765	-0.194
<i>Sisymbrium altissimum</i>	N	0.115	-0.765
<i>Sisymbrium loeselii</i>	I	-0.785	-0.538
<i>Solidago canadensis</i>	I	1.633	-0.417
<i>Telekia speciosa</i>	I	-0.085	-0.183
<i>Trifolium hybridum</i>	N	0.188	0.025
<i>Xanthium albinum</i>	N	-0.056	-0.044

**Table 6:** Mean indices of plant-soil feedback for each plant species. Status – invasive (I), non-invasive (N), Feedback index (germination) – index based on germination of seeds, Feedback index (biomass) – index based on plant biomass, x – no data for these species.



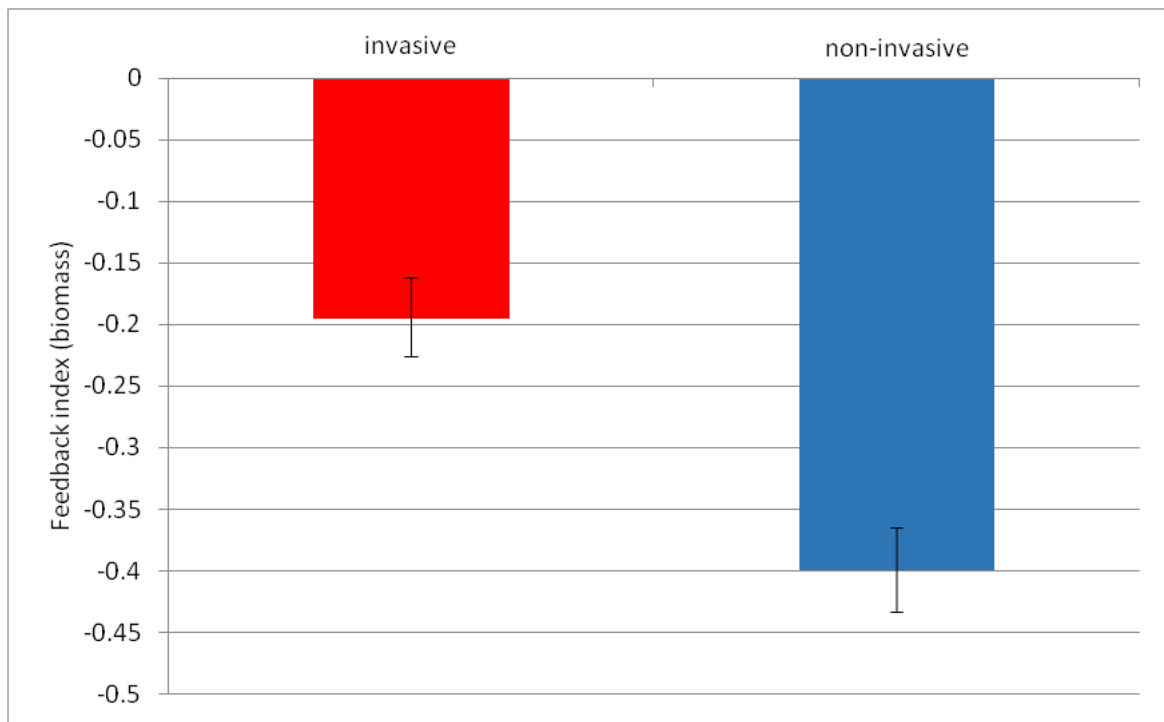
**Fig. 9: Feedback index based on seed germination in invasive and non-invasive species.** Invasive species had significantly higher index than non-invasive ( $p = 0.023$ ). The columns are means  $\pm$  SE.



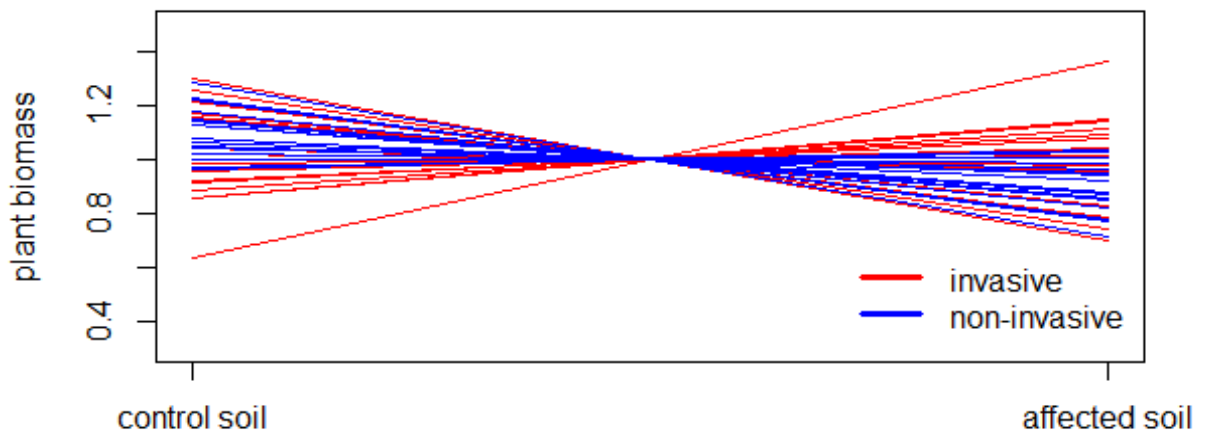
**Fig. 10: Germination of seeds in conditioned and control soil in invasive and non-invasive species.** On the left side is mean number of seedlings of each species in control soil and on the right side is mean number of seedlings of each species in conditioned soil. Values were standardized by species.



Feedback index based on plant biomass differ marginally significantly between invasive and non-invasive plant species ( $\chi^2(1)=2.883$ ,  $p=0.090$ ) (Table 5). Invasive plants had higher feedback index than non-invasive plants (Fig. 11 and 12). Mean index of plant-soil feedback for each plant species is shown in Table 6.



**Fig. 11: Feedback index based on plant biomass in invasive and non-invasive species.** Invasive species had marginally significantly higher index than non-invasive ( $p = 0.09$ ). The columns are means  $\pm$  SE.



**Fig. 12: Biomass of plant in conditioned and control soil in invasive and non-invasive species.** On the left side is mean biomass of each species in control soil and on the right side is mean biomass of each species in conditioned soil. Values were standardized by species.

Feedback index based on root-shoot ratio did not differ between invasive and non-invasive species ( $\chi^2(1)=0.607$ ,  $p=0.436$ ) (Table 5).

Feedback index based on production of seeds (number of seeds) did not differ between invasive and non-invasive species ( $\chi^2(1)=0.553$ ,  $p=0.457$ ) (Table 5).

### 3.3. Intraspecific plant-soil feedback and other traits

I tested each plant trait to compare what is better explanation of plant invasiveness. There were three significant traits – length-width ratio of propagule ( $p = 0.046$ ), height of plant (0.027) and specific leaf area ( $p = 0.032$ ) (Table 7). These three traits had also the biggest difference in AIC values (AIC of null model minus AIC of trait model). Indices based on germination and biomass were not significant, but they closely followed these three traits in delta AIC.

trait	p-value	$\Delta$ AIC
prop length:width ratio	<b>0.046</b>	<b>4.79</b>
height of plant	<b>0.027</b>	<b>4.38</b>
SLA	<b>0.032</b>	<b>4.07</b>
index biomass	0.158	0.00
index germination	0.260	-0.56
seedling RGR	0.322	-0.97
propagule weight	0.495	-1.07
first	0.409	-1.30
seedling establishment	0.499	-1.53
life history	0.331	-1.79
flower end	0.706	-1.86
flower start	0.716	-1.87
ploidy	0.746	-1.89
germinaton	0.110	-1.89
propagules number	0.892	-1.98
genome size	0.949	-2.00
dispersal	0.546	-3.87

**Table 7:** Importance of different traits in explaining species invasiveness. Significant results are shown in bold.  $\Delta$  AIC is counted as (AIC of null model minus AIC of trait model). Traits are sorted by  $\Delta$  AIC from higher to lower.

## 4. DISCUSSION

### 4.1. Methods

The experiment explored if the intraspecific plant-soil feedback can be used as a mechanism underlying plant invasiveness. I used the set of 34 neophytes of Czech Republic, of which some are invasive and some naturalized, but non-invasive (Pysek et al., 2012; Richardson et al., 2000). These species are from different taxonomic groups. There was also possibility to use pairs of closely related invasive and naturalized non-invasive species. While such a methodology has been used as a powerful tool in many previous studies on various topics (Goodwin et al., 1999; Mandak, 2003; Ugoletti et al., 2011), this approach was not used here as only too few pairs could be selected from our flora. Using bigger set of species is better in covering larger range of species of both types (invasive and naturalized, but non-invasive) and thus in generalization of the results on bigger set of invasive plants (van Kleunen et al., 2014). Using this particular set of species also bring several other advantages (see 2.1. Selection of plants and seed collection).

On the other hand, using the big set of species brings some constraints because species are not independent in terms of evolution history – they are more or less related (Felsenstein, 1985). In other words, the intensity of plant-soil feedback can be phylogenetically constrained. To understand the possible effects of phylogenetic relatedness on the results, it would be useful to include phylogenetic identity reconstructed using some database of phylogeny (for example DAPHNE phylogeny) among the tested traits. One could calculate phylogenetically independent contrasts and include them into analyses. By comparing the analyses with the phylogenetic independent contrasts it is possible to assess the importance of phylogenetic relationships between species for the observed patterns. I did not perform such an analysis, but it is next possible step to analyze my data. We plan to do it after adding further species to this dataset after this field season. It was recently suggested that plants belonging to different clades might show different patterns of plant-soil feedback. For example, Anacker et al. (2014) showed that 57 plant species from an old-field community exhibit phylogenetic signal in response to intraspecific feedback. This result is in agreement with findings of other studies (Brandt et al., 2009; Muenzbergova and Surinova, 2015) which showed that closely related species react similarly to soil of various origins. It can mean that for example invasive species perform better in soil of more distantly related species, as

closely related species share similar pathogens (Sweet and Burns, 2017). Also Pysek et al. (2009) showed that for predicting species invasiveness phylogenetic relatedness is important mainly on species level and for higher taxonomic levels it is not so important.

The next point which should be discussed is the duration of conditioning phase (and also the feedback phase). Conditioning phase of 12 weeks has been commonly used in a range of other previous studies (Meijer et al., 2011; van de Voorde et al., 2011; van Grunsven et al., 2007; van Grunsven et al., 2010). Also, the experiment of Vondráková (2016) showed that after ten weeks of conditioning the effect of plant-soil feedback was very similar. Thus, I decided to use conditioning of 12 weeks as plants were planted from the seedlings. Moreover, some plants started to flower and die at the end of the twelfth week. If the time of conditioning was too short, the plants would not have enough time to create specific soil community, thus the soil would not be conditioned.

Soil used in the experiment was prepared during the first year (May 2015) by mixing common garden soil and sand in the ratio 1:1. This mixture should be a good compromise to fulfill ecological requirements of the broad list of selected species. The advantage of prepared soil is that it has the same biotic and abiotic characteristics, and moreover, the researcher has a control which plants are actually affecting the soil (Brinkman et al., 2010). Another possibility is to collect soil from field populations of the plant and use this soil directly for the feedback phase. But this soil can differ in structure and chemical composition from the control. In this case soil from field can be also used as control but it has to be sterilized. But sterilization can change content of nutrients (Berns et al., 2008; He and Cui, 2009; Razavi darbar and Lakzian, 2007), pH (Mahmood et al., 2014; Razavi darbar and Lakzian, 2007) or aggregation of soil (Egli et al., 2006). Sterilization can also influence biomass of plants which grow in it (He and Cui, 2009), thus using sterilization of soil in plant-soil feedback experiments can influence the results. Also if the soil is sterilized it is not possible to examine the influence of abiotic part of soil on plant.

The effects of soil storage on soil communities have been extensively discussed (Gould and Liberta, 1981; Miller et al., 1985; Visser et al., 1984; Wick et al., 2009). It has been suggested that the largest difference is between intact and disturbed soil. When already disturbed, time of storage has little effect on soil communities as long as the soil is dry and is stored for a few years only. Additionally, an important assumption, namely that the composition of the soil used for planting one population of plant is identical, is fulfilled, because soil used for cultivation and control of the specific species was taken at the same

time. The fact that the soil can slightly differ between years is marginal and irrelevant for the experiment.

I also considered buying commercial growing substrate, but it is in fact known to be very heterogeneous even between different packs of the same brand bought at the same time (Bukovska unpubl.). Also it was not able to store the soil in the fridge because of its high volume.

I decided to use uncultivated soil exposed to the same conditions as the conditioned soil as control. Such an approach was used also in a range of previous studies (Kardol et al., 2007; Kulmatiski et al., 2011; Perkins and Nowak, 2013; Wang et al., 2013). Alternatively, it is also possible using soil cultivated by a native species or by native community as a control. Selecting such a species or community is, however, not straightforward, as each of the model species is occurring in different habitat affecting different native species and optimal controls would thus differ between species. Importantly, response of each species to its soil will be compared to its response to the same control. Thus exact identity of the control will not change the results as long as the controls are comparable between species.

## 4.2. The experiment

The aim of my experiment was to test the hypothesis that intraspecific plant-soil feedback can be used as an explanation of plant invasiveness (Callaway et al., 2004; Klironomos, 2002; Morrien and van der Putten, 2013; van Grunsven et al., 2010). As the first step to find if there is difference in plant-soil feedback between invasive and non-invasive plants, I examined intraspecific plant-soil feedback of each species separately. According to the hypothesis, invasive plants should perform better in conditioned soil than in control. The results vary between different measurements of plant performance.

Better performance in their own soil was found in *Asclepias syriaca* and *Conyza canadensis*. These two species had higher number of seedlings and higher biomass in the conditioned soil. As both species are invasive, this positive feedback can be the mechanism underlying their invasiveness as was demonstrated for *Bromus tectorum* (Morris et al., 2016), *Impatiens glandulifera* (Pattison et al., 2016) or *Lespedeza cuneata* (Coykendall and Houseman, 2014). If they influence the soil in their favor, they grow and germinate better and are able to spread and become invasive. Additionally, other three invasive species showed higher germination in conditioned soil supporting the idea that higher germination induced by

changes in own soil may be the reason of plant invasiveness. Higher germination in own soil could be advantage in the time of forming the community of plants, when seeds of these species are supported by self-conditioned soil. This can be advantage mainly for annuals, which have to grow from seeds every year thus self-conditioned soil supports the germination of the plants. However, only two out of five invasive species having higher germination in conditioned soil were annuals. Three other species were perennials. Even though there are many studies on plant-soil feedback and invasiveness of plants (Agrawal et al., 2005; Birnbaum and Leishman, 2013; Callaway et al., 2004; Wang et al., 2017), just few of them also explored effects on seed germination by changing in soil and these studies show negative, positive and no effect on germination of seeds in conditioned soil (Beckstead and Parker, 2003; de la Pena et al., 2010; Muenzbergova and Surinova, 2015).

Except germination there were many significant tests with biomass of plant. Only two species grew better in their own soil as I wrote before (*A. syriaca* and *C. canadensis*). Other eleven species had higher biomass in control soil suggesting that they experience negative intraspecific plant-soil feedback. It is in agreement with many previous studies that claim that most plants have negative intraspecific plant soil feedback (Bever, 2003; Kulmatiski et al., 2008; van der Putten et al., 2013). These species were both invasive and non-invasive. But the fact that the feedback is not positive for all invasive species does not mean that it cannot be the mechanism underlying plant-invasiveness as invasive species maybe experience just less negative feedback than non-invasive ones. For example *Alliaria petiolata* inhibits mycorrhizal fungal mutualists in the soil thus suppress the growth of other plant species which rely on these symbionts (Callaway et al., 2008). The negative effect of this inhibition influence also *A. petiolata* itself but not in such extent as other species, so *A. petiolata* can spread and potentially become invasive.

To see what role the depletion of nutrients plays in plant-soil feedback of individual species, I compared biomass of plants from the first phase with the biomass from the second phase. The tests for nutrient depletion during the first phase were significant for four species. Only *Abutilon theophrasti* showed positive relationship between biomass of the first and second phase. It means that the bigger it was in the first phase the bigger it was in the second phase. Depletion of nutrients thus had no influence on performance of this species. Despite this fact, non-invasive *A. theophrasti* showed negative intraspecific plant-soil feedback (had lower plant biomass in own soil) suggesting that it can be caused by some pathogens accumulated in soil, which can prevent the plant from becoming invasive.

On the other hand the relationship between biomass of the first and second phase was negative for *Impatiens parviflora*, *Chenopodium strictum* and *Rumex alpinus*. As they performed worse in their own soil, i.e. they experienced negative intraspecific plant-soil feedback, it could be caused by depletion of nutrients in the first phase. Although *I. parviflora* and *R. alpinus* are invasive, they do not experience positive intraspecific plant-soil feedback thus they suppress their own growth in self-conditioned soil. Plant-soil feedback is probably not the mechanism underlying their invasiveness or at least they do not build positive feedback with soil during one growing season (Day et al., 2015).

The relationship between root-shoot ratio and plant-soil feedback is more complicated to interpret than biomass or germination of seeds and also there are not many studies using this variable (but see (Bever, 1994; Meijer et al., 2011)). Higher root-shoot ratio in conditioned soil means that the plant allocates more to the roots in conditioned than in control soil a vice versa. Lower biomass of roots in conditioned soil can for example mean that the plant accumulates arbuscular mycorrhizal fungi which can help to the plant to become dominant as in case of C4 tall grasses in tall-grass prairie (Hartnett and Wilson, 1999). Because of that connection the plant may create fewer roots than in unconditioned soil. Lower biomass of roots in conditioned soil may be connected also with negative feedback. For example plant roots may be suppressed by soil pathogens or herbivores accumulated in soil and thus plant has lower root biomass.

In the experiment the values of root-shoot ratio vary among different species and are not consistent with plant statuses of invasiveness. It indicates that plant-soil feedback based on root-shoot ratio probably does not differ between invasive and non-invasive plants and is probably species specific.

Only two non-invasive species showed significant difference in production of seeds – *Amaranthus albus* and *Chenopodium strictum*. Both of them produced more seeds in conditioned soil. As both species experienced negative plant-soil feedback (biomass) with own soil this is probably the consequence of this relationship – if the plant grows worse in conditioned soil it probably also produces fewer seeds than in control soil.

Although some invasive species showed positive feedback with soil, many of them did not react on the conditioning of soil. It could be caused by the type of the experiment. The experiment was realized on many species to reveal general difference in plant-soil feedback between invasive and non-invasive neophytes of the Czech Republic. For that reason, it was not possible to study every single species as precisely as in case of studies on only one or few species (van Kleunen et al., 2014). For example species differ in growth optima and are



usually associated with limited number of habitats. As the number of species in the experiment was high it was not possible to grow them under their natural conditions especially when I wanted to compare them. All species were thus planted under the same conditions which were more or less suitable for all species. However it is not the mistake to use one set of environmental conditions, but it is necessary to take it into account as the results may be valid only for these conditions.

Different results in plant-soil feedback could also occur after longer time of cultivation. However because it was shown that after ten weeks the composition of soil varies only a little (Vondráková, 2016), this study was not focused on the changes of plant-soil feedback during next growing seasons (Day et al., 2015). Differences which can occur in plant-soil feedback after long-term invasion were shown in studies on *Heracleum mantegazzianum* (Dostal et al., 2013) and *Vicia vilosa* (Lau and Suwa, 2016). However, in these studies the soil was not cultivated. They collected soil from sites with different time of species invasion and compared plant performance in these soils. Both species experienced more negative intraspecific plant-soil feedback in soils with long-term period of invasion, probably because the negative plant-soil feedback is likely to develop slowly.

To statistically verify the hypothesis that positive intraspecific plant-soil feedback could be the mechanism underlying plant invasiveness I compared feedback indices of each species within their status. Feedback index based on germination differed significantly between invasive and naturalized, but non-invasive, species. Invasive species had higher indices than non-invasive ones; it means that they germinated better in their own soil. It supports the hypothesis suggesting that invasive plants germinate better if they have seeds in soil with con-specific history thus the positive feedback with soil may be the mechanism underlying their invasiveness. However, study on invasive *Ammophila arenaria* (Beckstead and Parker, 2003) showed that this species does not germinate better in the own soil compared to sterilized soil. Also study on *Carpobrotus edulis* (de la Pena et al., 2010), invasive species in Mediterranean Europe, showed no effect of soil conditioning on seed germination. But these two studies were done only on single specific invasive species and did not compare germination of invasive species with non-invasive. My experiment brings data about relationship of seed germination and plant-soil feedback for many species which is unique. As there are not many studies focusing on influence of plant-soil feedback on seed germination, these results can bring an important piece of knowledge to the mechanisms of plant invasiveness.

Feedback index based on plant biomass was marginally significant in the way that invasive species performed better in their own soil compared to non-invasive ones. It is in agreement with many previous studies, but these studies have been made only for one or few invasive species (Coykendall and Houseman, 2014; Emam et al., 2014; Klironomos, 2002; Kulmatiski et al., 2008; Levine et al., 2006; Nijjer et al., 2008; van Grunsven et al., 2007).

Comparisons of root-shoot ratio and production of seeds between invasive and non-invasive plants were not significant. Insignificance of the test with root-shoot ratio is in coincidence with single tests of root-shoot ratio within species as these results vary inconsistently. On the other hand, the non significant result of production of seeds can be caused by small dataset as we had data on production of seeds just for six species. As plant-soil feedback is usually studied using only plant biomass I cannot compare this result with other studies. But collecting new data on production of seeds in own versus control soil can bring a new insight to the mechanism of plant-soil feedback.

From these results it is clear that intraspecific plant-soil feedback can vary between different stages of plant lifecycle (Muenzbergova and Surinova, 2015). According to my results the most important is influence of plant-soil feedback on germination of seeds followed by influence on plant biomass. Because invasive plants germinated and grew better in own soil (in soil with con-specific history) than non-invasive ones it can be the mechanism underlying their invasiveness. Although these results are positive it is necessary to compare it with other plant traits to fully understand the importance of intraspecific plant-soil feedback for plant invasiveness.

To test the relative importance of plant-soil feedback for species invasiveness, data on wide range of additional traits were compiled for the studied species including also feedback indices based on germination and plant biomass and SLA – traits were measured within the experiment. Invasiveness of plants was best explained by propagule length-width ratio, height of plant and plant SLA. Difference between invasive and non-invasive naturalized species for propagule length-width ratio was also shown in study of Moravcova et al. (2010) from which this trait was actually taken over. This trait means that the lower the ratio is the more rounded the propagules are. Invasive plants have lower propagule ratio thus they have more rounded propagules which can be easily spread by water or wind and thus it helps them to become invasive. It is in connection with weight of propagules but this trait was not significant in the experiment. Height of plant and SLA significantly differ between invasive and non-invasive plants as in study of van Kleunen et al. (2010). Invasive plants are higher and have higher SLA as both traits are associated with high plant performance.

Intraspecific plant-soil feedback was a better predictor based on delta AIC than many other traits. It suggests that although these traits are not significant they are still more important than many other traits. For example plant-soil feedback is a better predictor than ploidy or genome size. But negative result of genome size is in coincidence with another study (Kubesova et al., 2010) which shows that invasive plants differ from their non-invasive congeners having smaller genomes but not from naturalized, but non-invasive, species. It may mean that this trait brings advantage to plant at the stage of naturalization but not in the final stage of invasion. Also, meta-analysis of van Kleunen et al. (2010) shows that more significant differences in trait were between invasive vs. native plants.

Although, intraspecific plant-soil feedback was significant only for germination of seeds and not for plant biomass and it was not the best explanation of plant invasiveness in the experiment, measuring of feedbacks of further species can bring more light into the clarification of mechanism of plant invasiveness.

## 5. CONCLUSION

The aim of this study was to test the hypothesis that intraspecific plant-soil feedback can be used as an explanation of plant invasiveness.

Single species showed both positive and negative feedback with soil. Although some invasive species supported germination of seeds by changes in the soil, growth and thus biomass was higher in the cultivated soil only in two of them. This suggests that intraspecific plant-soil feedback can vary between different life stages of the plants.

In the overall tests of difference of the effect of intraspecific plant-soil feedback between invasive and non-invasive plants I demonstrated that invasive species show more positive (germination) or at least less negative (biomass) intraspecific plant-soil feedback than non-invasive species. It means that intraspecific plant-soil feedback could be the mechanism underlying their invasiveness.

To explore the relative importance of intraspecific plant-soil feedback for species invasiveness I compared it with wide range of additional traits. Although intraspecific plant-soil feedback was not the best predictor of invasiveness, it was better than many other traits previously used to explain species invasiveness (for example genome size, ploidy or type of dispersal).

My results suggest that invasive species may alter the soil in their favor thus experience positive intraspecific plant-soil feedback and that this feedback could be the mechanism underlying their invasiveness. These results may improve our understanding of invasive mechanism of plants so we can better predict which species are potentially invasive.

Future studies will focus on obtaining more data on intraspecific plant-soil feedback for invasive and non-invasive neophytes of the Czech Republic so there will be more power to generalize the results. There are also some interesting directions of future plant-soil feedback research for example the difference between feedback with soil in different life stages or detection of microbial community in the soil and its changes in connection with invasive plants.

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## 7. APPENDICES

**APPENDIX 1:** List of species used in the study. Shortcut – shortcut of name of species. Status – invasive (I) and non-invasive (N). Locality – number of localities from which seeds were collected (Planta Naturalis is counted also as a locality). Year – year when species were planted (due to higher number of localities, some species were planted both years).

species	shortcut	family	status	lifeform	seed origin	locality	year
<i>Abutilon theophrasti</i> Med.	<i>Abu.the</i>	<i>Malvaceae</i>	N	annual	Planta Naturalis	1	2016
<i>Amaranthus albus</i> L.	<i>Ama.alb</i>	<i>Amaranthaceae</i>	N	annual	natural populations	1	2016
<i>Amaranthus powellii</i> S. Watson	<i>Ama.pow</i>	<i>Amaranthaceae</i>	I	annual	natural populations	3	2015/16
<i>Amaranthus retroflexus</i> L.	<i>Ama.ret</i>	<i>Amaranthaceae</i>	I	annual	natural populations	3	2016
<i>Ambrosia artemisiifolia</i> L.	<i>Amb.art</i>	<i>Asteraceae</i>	I	annual	Planta Naturalis	1	2016
<i>Asclepias syriaca</i> L.	<i>Asc.syr</i>	<i>Apocynaceae</i>	I	perennial	natural populations	3	2016
<i>Bidens frondosa</i> L.	<i>Bid.fro</i>	<i>Asteraceae</i>	I	annual	natural populations	2	2015/16
<i>Cannabis ruderalis</i> Janisch.	<i>Can.rud</i>	<i>Cannabaceae</i>	I	annual	natural populations	2	2016
<i>Conyza canadensis</i> (L.) Cronq.	<i>Con.can</i>	<i>Asteraceae</i>	I	annual	natural populations	1	2015
<i>Datura stramonium</i> L.	<i>Dat.str</i>	<i>Solanaceae</i>	N	annual	natural populations	1	2015
<i>Digitalis purpurea</i> L.	<i>Dig.pur</i>	<i>Plantaginaceae</i>	N	perennial	natural populations	3	2015/16
<i>Echinops sphaerocephalus</i> L.	<i>Echi.sph</i>	<i>Asteraceae</i>	I	perennial	natural populations	2	2015
<i>Erigeron annuus</i> (L.) Pers.	<i>Eri.ann</i>	<i>Asteraceae</i>	I	annual	Planta Naturalis	1	2016
<i>Galinsoga parviflora</i> Cav.	<i>Gal.par</i>	<i>Asteraceae</i>	I	annual	natural populations	1	2016
<i>Geranium pyrenaicum</i> Burm. fil.	<i>Ger.pyr</i>	<i>Geraniaceae</i>	N	perennial	natural populations	1	2016

species	shortcut	family	status	lifeform	seed origin	locality	year
<i>Hesperis matronalis</i> L.	<i>Hes.mat</i>	<i>Brassicaceae</i>	N	perennial	natural populations	1	2016
<i>Chenopodium strictum</i> Roth	<i>Chen.str</i>	<i>Amaranthaceae</i>	N	annual	natural populations	3	2015/16
<i>Impatiens parviflora</i> DC.	<i>Imp.par</i>	<i>Balsaminaceae</i>	I	annual	natural populations	1	2015
<i>Lupinus polyphyllus</i> Lindl.	<i>Lup.pol</i>	<i>Fabaceae</i>	I	perennial	Planta Natur. + natural population	2	2016
<i>Lychnis coronaria</i> (L.) Desr.	<i>Lych.cor</i>	<i>Caryophyllaceae</i>	N	perennial	natural populations	3	2015/16
<i>Medicago sativa</i> L.	<i>Med.sat</i>	<i>Fabaceae</i>	N	perennial	natural populations	2	2015/16
<i>Mimulus guttatus</i> DC.	<i>Mim.gut</i>	<i>Phrymaceae</i>	N	perennial	Planta Naturalis	1	2016
<i>Oenothera biennis</i> L.	<i>Oen.bie</i>	<i>Onagraceae</i>	N	biennial	natural populations	2	2015/16
<i>Oenothera glazioviana</i> M. Micheli	<i>Oen.gla</i>	<i>Onagraceae</i>	N	biennial	natural populations	2	2015/16
<i>Rudbeckia hirta</i> L.	<i>Rud.hir</i>	<i>Asteraceae</i>	N	perennial	natural populations	1	2015
<i>Rudbeckia laciniata</i> L.	<i>Rud.lac</i>	<i>Asteraceae</i>	I	perennial	natural populations	2	2015/16
<i>Rumex alpinus</i> L.	<i>Rum.alp</i>	<i>Polygonaceae</i>	I	perennial	natural populations	3	2015
<i>Rumex thyrsiflorus</i> Fingerh.	<i>Rum.thy</i>	<i>Polygonaceae</i>	N	perennial	natural populations	3	2016
<i>Sisymbrium altissimum</i> L.	<i>Sis.alt</i>	<i>Brassicaceae</i>	N	annual	Planta Naturalis	1	2016
<i>Sisymbrium loeselii</i> L.	<i>Sis.loe</i>	<i>Brassicaceae</i>	I	annual	natural populations	2	2015/16
<i>Solidago canadensis</i> L.	<i>Sol.can</i>	<i>Asteraceae</i>	I	perennial	natural populations	1	2015
<i>Telekia speciosa</i> (Schreber) Baumg.	<i>Tel.spe</i>	<i>Asteraceae</i>	I	perennial	natural populations	3	2015/16
<i>Trifolium hybridum</i> L.	<i>Tri.hyb</i>	<i>Fabaceae</i>	N	perennial	Planta Naturalis	1	2016
<i>Xanthium albinum</i> (Widd.) H. Scholtz et Sukop	<i>Xan.alb</i>	<i>Asteraceae</i>	N	annual	Planta Naturalis	1	2016

**APPENDIX 2:** Correlation matrix of plant traits used in the study (Pearson correlation coefficient).

	FI (biomass)	FI (germination)	SLA	First report	Life history	Height of plant	Dispersal type	Flower start	Flower end
FI (biomass)	1	0.22	0.5	-0.07	-0.01	0.15	0.26	0.08	-0.1
FI (germination)	0.22	1	0.25	-0.25	0.04	0.16	0.09	0.12	0.14
SLA	0.5	0.25	1	0.16	-0.15	-0.05	0.1	0.15	-0.02
First report	-0.07	-0.25	0.16	1	-0.07	0.18	-0.02	0.4	0.16
Life history	-0.01	0.04	-0.15	-0.07	1	0.16	0.19	-0.11	-0.35
Height of plant	0.15	0.16	-0.05	0.18	0.16	1	0.14	0.43	-0.04
Dispersal type	0.26	0.09	0.1	-0.02	0.19	0.14	1	0.42	-0.07
Flower start	0.08	0.12	0.15	0.4	-0.11	0.43	0.42	1	0.32
Flower end	-0.1	0.14	-0.02	0.16	-0.35	-0.04	-0.07	0.32	1
Genome size	0	-0.04	-0.35	0.13	0.33	0.28	0.33	0.21	-0.06
Ploidy	-0.04	0.09	-0.16	0.13	0.12	0.15	0.07	0.27	0.09
Gemination	-0.08	0.05	0.1	-0.17	-0.12	0.11	0.06	-0.17	-0.22
Seedling RGR	0.08	0.2	0.27	0.04	-0.09	-0.18	0.43	0.05	0.28
Seedling est.	-0.04	-0.17	-0.12	-0.11	0.29	0.24	-0.02	-0.01	-0.38
N of propagules	-0.01	0.3	0.2	-0.32	-0.32	-0.09	0.1	0	0.15
Propagule size	0.28	0.14	0.09	0.03	0.46	0.27	0.4	0.06	-0.14
Propagule weight	0.12	-0.05	-0.13	0.04	-0.12	0.15	-0.25	0.3	0.23

	Genome size	Ploidy	Gemination	Seedling RGR	Seedling est.	N of propagules	Propagule size	Propagule weight
FI (biomass)	0	-0.04	-0.08	0.08	-0.04	-0.01	0.28	0.12
FI (germination)	-0.04	0.09	0.05	0.2	-0.17	0.3	0.14	-0.05
SLA	-0.35	-0.16	0.1	0.27	-0.12	0.2	0.09	-0.13
First report	0.13	0.13	-0.17	0.04	-0.11	-0.32	0.03	0.04
Life history	0.33	0.12	-0.12	-0.09	0.29	-0.32	0.46	-0.12
Height of plant	0.28	0.15	0.11	-0.18	0.24	-0.09	0.27	0.15
Dispersal type	0.33	0.07	0.06	0.43	-0.02	0.1	0.4	-0.25
Flower start	0.21	0.27	-0.17	0.05	-0.01	0	0.06	0.3
Flower end	-0.06	0.09	-0.22	0.28	-0.38	0.15	-0.14	0.23
Genome size	1	0.47	-0.07	-0.12	0.02	-0.28	0.26	0.04
Ploidy	0.47	1	-0.12	-0.04	-0.04	0	0.06	0.08
Gemination	-0.07	-0.12	1	0.4	0.13	-0.03	-0.11	-0.36
Seedling RGR	-0.12	-0.04	0.4	1	-0.28	0.22	0.12	-0.28
Seedling est.	0.02	-0.04	0.13	-0.28	1	-0.12	0.05	0.17
N of propagules	-0.28	0	-0.03	0.22	-0.12	1	-0.12	-0.16
Propagule size	0.26	0.06	-0.11	0.12	0.05	-0.12	1	-0.04
Propagule weight	0.04	0.08	-0.36	-0.28	0.17	-0.16	-0.04	1

**APPENDIX 3:** Detailed explanation of individual traits used in the study.

**Feedback index based on germination (FI – germination):**

Index of intraspecific plant-soil feedback counted from data on germination of individual species from the experiment. The pattern is written in the methodology.

**Feedback index based on biomass (FI – biomass):**

Index of intraspecific plant-soil feedback counted from data on biomass of individual species from the experiment. The pattern is written in the methodology.

**SLA:**

Specific leaf area ( $\text{g}/\text{cm}^3$ ) counted with using data from the experiment. The pattern is written in the methodology.

**First report:**

First reported occurrence of the species in the wild in the Czech Republic. (Pysek et al., 2012)

**Life history:**

There were three groups of different life history in my dataset – annual, biennial and perennial. As only two species were biennial and all other species were annual or perennial, biennial were merged with annual to one group. (Pysek et al., 2012)

**Height of plant:**

Releasing height of plant measured in meters. (LEDA database)

**Dispersal type:**

For each species the most common type of dispersal was chosen. Types of dispersal were: hemerochory, zoochory, nautochory, autochory. (LEDA database)

**Flower start:**

Start of flowering of each species. (Kubát, 2002)

**Flower end:**

End of flowering of each species (Kubát, 2002)

**Genome size:**

Mean holoploid genome size. (Kubesova et al., 2010)

**Ploidy:**

Level of ploidy. (Kubesova et al., 2010)

**Germination:**

Germination of seeds which were freshly harvested, dry-stored for one month or cold-stratified and then germinated under different temperatures. (Moravcova et al., 2010)

**Seedling RGR:**

Seedling relative growth rate ( $\text{g} \cdot \text{g}^{-1} \cdot \text{day}^{-1}$ ). (Moravcova et al., 2010)

**Number of propagules:**

The average number of propagules per single plant, or single shoot of a clonal species. (Moravcova et al., 2010)

**Propagule size:**

The length-width ratio of propagules. This index describes the two-dimensional “shape” of the propagule, with low values characterizing rounded propagules. (Moravcova et al., 2010)

**Propagule weight:**

Mean weight of propagule of each species (g). (Moravcova et al., 2010)