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Interaction of plants and soil and other factors affecting plant invasiveness

Interakce rostlin a půdy a další faktory ovlivňující invazivnost rostlin

Ph.D. Thesis / Disertační práce

Supervisor / Školitel: doc. RNDr. Zuzana Münzbergová, Ph.D.

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Declaration

Hereby I declare that I did the present Ph.D. thesis by myself with the use of sources cited. The papers that form part of the thesis were co-authored and author contributions are stated in the proper section. I have not used any part of this thesis for claiming any other academic title.

In Prague, 10th June 2019 Anna Aldorfová

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Abstract

Plant invasions represent a major ecological and socio-economical issue and understanding the drivers as well as consequences of plant invasions is thus one of the main goals of plant ecology. It is equally important to reveal general patterns underlying plant invasions and to understand the details of biology of individual invaders. In this thesis I explored plant-soil feedback (PSF) as a possible general mechanism underlying plant invasiveness, and also focused in detail on drivers and consequences of *Impatiens parviflora* invasion.

The aims of this thesis were to i) assess the differences in intraspecific PSF between invasive and alien non-invasive species using a large set of species; ii) explore the relationship between PSF, residence time and phylogenetic novelty of the alien species; iii) compare the importance of PSF and other plant characteristics for plant invasiveness; iv) compare PSF between invasive and native congeners of similar level of dominance in the field; v) evaluate the effect of cultivating conditions on results of PSF experiments; vi) describe invasion dynamics and determine factors affecting spread of invasive *I. parviflora* using a method of monitoring its natural spread in several types of habitats, and vii) assess the impact of *I. parviflora* on native vegetation of oak-hornbeam forests using a removal experiment.

The results of the thesis may be summarized as follows: i) Invasive species have more positive PSF for seedling establishment, but not for biomass of adult plants, than alien non-invasive species; ii) Phylogenetically novel species have more positive PSF than species with a native congener, suggesting greater release from natural enemies. The relationship between PSF and residence time differs between invasive and alien non-invasive species, indicating that individual alien species differ in the extent of enemy release or subsequent accumulation of local pathogens; iii) PSF for seedling establishment belongs to the best predictors of invasive status for our set of species, following specific leaf area, height, residence time, and seedling growth rate; iv) Under standard conditions, invasive species do not differ from native dominants in terms of PSF; v) Cultivating conditions have substantial effect on results of PSF experiments. Individual stages of plant lives, as well as invasive and native dominants, respond differently to the conditions, suggesting that different processes underlie the feedback; vi) *I. parviflora* spreads most easily through oak-hornbeam forests, followed by acidophilous oak and mixed coniferous forests, but is able to penetrate dry grasslands on rocks and termophilous oak forests as well. Most important factors determining its spread are cover of herb layer, soil moisture and canopy openness, but individual stages of *I. parviflora* are affected by them to different extent;

vii) *I. parviflora* has weak, yet significant negative impact on native vegetation of oak-hornbeam forests, suppressing mostly small, early flowering species.

Overall, I showed that PSF is one of the mechanisms distinguishing between invasive and alien non-invasive plants. However, further investigations are needed to understand the processes underlying the feedback and reveal plant traits determining it. I pointed to the importance of studying some overlooked aspects of PSF such as role of cultivating conditions or inclusion of multiple phases of life cycle. The thesis illustrated that monitoring natural spread of invasive species can provide important insights into the determinants of their distribution, and I showed that in case of *I. parviflora* the determinants depend on spatial scale studied and differ between life stages of the species.

Key words: alien plant species; cultivating conditions; impact of invasive species; natural spread observation; phylogenetic relatedness; plant functional traits; plant invasiveness; plant-soil interactions; removal experiment; residence time; small balsam (*Impatiens parviflora*).

Abstrakt (in Czech)

Rostlinné invaze představují významný ekologický a socio-economický problém, studium faktorů podporujících invaze a studium následků invazí je proto jedním z hlavních cílů rostlinné ekologie. Důležité je jak porozumět obecným zákonitostem rostlinných invazí, tak detailně poznat biologii jednotlivých invazních druhů. V této práci jsem se zaměřila na vnitrodruhovou zpětnou vazbu mezi rostlinou a půdou (tzv. plant-soil feedback, dále PSF) jako na jeden z možných mechanismů podmiňujících invazivnost rostlin obecně a na příčiny a následky invaze modelového druhu netýkavka malokvětá (*Impatiens parviflora*).

Cíle předkládané disertační práce byly: i) na velkém souboru druhů porovnat PSF mezi invazními a nepůvodními, ale neinvazními druhy rostlin; ii) prozkoumat vztah mezi PSF, dobou od zavlečení nepůvodních druhů a mírou jejich fylogenetické příbuznosti s původní florou; iii) porovnat význam PSF a dalších druhových vlastností pro invazivnost rostlin; iv) porovnat vnitrodruhový PSF invazních a původních dominant; v) vyhodnotit vliv kultivačních podmínek na výsledky PSF pokusů; vi) popsat dynamiku a faktory ovlivňující šíření invazního druhu *I. parviflora* pomocí sledování jeho přirozeného šíření v několika typech habitatů; vii) zhodnotit vliv *I. parviflora* na původní vegetaci dubohabrových lesů pomocí vytrhávacího pokusu.

Výsledky práce se dají shrnout následovně: i) Invazní druhy mají kladnější PSF založený na vzcházení a přežívání semenáčků, ale ne PSF založený na biomase dospělých rostlin, než nepůvodní neinvazní druhy rostlin; ii) Druhy fylogeneticky méně příbuzné původní vegetaci mají kladnější PSF než druhy, které mají v původní floře blízkého příbuzného, což naznačuje, že se mohou efektivněji zbavit přirozených nepřátel. Invazní a nepůvodní neinvazní rostliny vykazují odlišný vztah mezi PSF a dobou od zavlečení, což naznačuje, že jednotlivé nepůvodní druhy se liší v míře, do jaké se zbaví přirozených nepřátel, nebo v rychlosti, s jakou následně akumulují místní patogeny; iii) PSF založený na vzcházení a přežívání semenáčků patří mezi nejlepší prediktory invazního statutu pro náš soubor nepůvodních druhů, následuje specifickou listovou plochu, výšku, dobu od zavlečení a růstovou rychlost; iv) PSF invazních druhů se ve standardních podmínkách neliší od PSF původních dominant; v) Kultivační podmínky mají významný vliv na výsledky PSF pokusů. Jednotlivá stadia životního cyklu rostlin se, stejně jako invazní a původní dominanty, odlišují v reakci na kultivační podmínky, což naznačuje, že jejich PSF je podmíněný různými mechanismy; vi) *I. parviflora* se nejsnáze šíří v dubohabrových lesích, následovaných kyselými doubravami a smíšenými lesy, je ale schopná proniknout i na skalní stepi nebo do teplomilných doubrav. Nejdůležitější faktory ovlivňující její šíření jsou pokryvnost bylinného patra, půdní vlhkost a míra zastínění stromovým patrem, ale jednotlivá stadia životního cyklu se liší citlivostí vůči jednotlivým faktorům; vii) *I. parviflora* má slabý negativní vliv na původní vegetaci a potlačuje zejména malé, časně kvetoucí druhy rostlin.

V této práci jsem ukázala, že PSF je jedním z mechanismů rozlišujících invazní a neinvazní nepůvodní druhy rostlin, další výzkum je ale potřeba, abychom porozumněli procesům, které PSF podmiňují, a abychom odhalili vlastnosti rostlin, které pomáhají určovat jeho intenzitu. Dále jsem poukázala na důležitost studia určitých dosud přehlížených aspektů PSF, jako je role kultivačních podmínek nebo zahrnutí různých stadií životního cyklu. Ukázala jsem, že sledování přirozeného šíření invazního druhu může výrazně pomoci porozumění jejich rozšíření a na příkladě *I. parviflora* jsem ukázala, že tyto faktory zaleží na studované škále a liší se mezi jednotlivými životními stadii druhu.

Klíčová slova: nepůvodní druhy rostlin; kultivační podmínky; vliv původních rostlin; pozorování přirozeného šíření; fylogenetická příbuznost; funkční vlastnosti rostlin; invazivnost rostlin; interakce mezi rostlinou a půdou; vytrhávací pokus; doba od zavlečení; netýkavka malokvětá (*Impatiens parviflora*).

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Introduction

Invasive species, i.e. alien species that are able to establish in the introduced region and massively spread there (Pysek et al. 2004), represent a major ecological and socio-economic problem in many parts of the world and are thus one of the key issues of ecological research in the last decades. Invasive plants affect natural communities, displace native species, change vegetation structure, cause reduction in diversity in the affected areas (Hejda et al. 2009; Powell et al. 2013), undermine the functioning of whole ecosystems (Richardson and Pysek 2012) and cause significant economic losses (Zavaleta 2000). Identifying future invaders, preventing emergence of new invasive species and assessing the impact of the existing ones is thus in the interest of our society and presents a challenge to both conservation and international commerce (Mack et al. 2000).

One of the main goals of invasive ecology is to assess alien plant invasiveness, i.e. their ability to become invasive (Rejmanek and Richardson 1996), and thus differentiate invasive species from their non-invasive relatives. Previous studies have identified many characteristics that seem to be important for plant invasiveness, such as high phenotypic plasticity (Daehler 2003), more efficient use of resources (Dick et al. 2014), high generative reproduction (e.g., Burns 2004; Burns et al. 2013; Moravcova et al. 2010), genome size and ploidy level (te Beest et al. 2012), high growth rate during the early stages of the life-cycle (e.g., Burns 2004; Leishman et al. 2014), or phylogenetic relatedness to native flora (Strauss et al. 2006). While all these characteristics 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. 2010b).

Another possible explanation of the success of invasive plant species suggested relatively recently is the feedback between the plants and the soil in which they grow, i.e. plant-soil feedback (e.g., Callaway et al. 2004; Kulmatiski et al. 2008; Levine et al. 2006; Reinhart and Callaway 2004; 2006; Suding et al. 2013). Plant-soil feedback (PSF) is a relationship in which plants affect the composition of the soil and such modified soil affects the growth of the plants (Bever 1994; Bever et al. 1997). Commonly, two types of feedbacks, intraspecific and interspecific, 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).

The importance of interspecific PSF for plant invasiveness has been known for quite some time. It is caused 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 (e.g., Del Fabbro and Prati 2015; Wardle et al. 1998). Similar effects caused by changes in composition of soil communities have been described relatively recently (e.g., 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. Invasive species as well as some native dominants can, however, alter the composition of the soil in their favor and thus show positive intraspecific PSF (Anacker et al. 2014; Klironomos 2002). As invasive species are commonly being dominant in their new environment (Hobbs et al. 2006), it can be expected that intraspecific positive PSF could be an important factor allowing the invasive species to achieve their dominant position and become invasive.

Positive (or less negative compared to natives) intraspecific feedback between invasive plant species and soil has been demonstrated for several invasive species (e.g., Callaway et al. 2004; Coykendall and Houseman 2014; Dostal et al. 2013; Gundale et al. 2014; Maron et al. 2014; Nijjer et al. 2008; van Grunsven et al. 2007; Yang et al. 2013) and has been also detected in reviews of Kulmatiski et al. (2008) and Meisner et al. (2014). The importance of positive intraspecific PSF for plant invasiveness has also been supported by a modeling study of Levine and D'Antonio (1999). More positive intraspecific feedbacks in invasive species compared to the native species has been traditionally explained by The Enemy Release Hypothesis (Elton 1958). When the invasive plants move 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 and D'Antonio 1999). While generalists can attack both the native and the 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 might lead to positive intraspecific feedback between the plant and the soil, have usually lower host specificity (Smith and Read 2008) and the invasive plants can thus profit from their accumulation (Yang et al. 2014).

The Enemy Release Hypothesis, however, cannot explain why some alien species become invasive while vast majority of them fails to (Williamson and Fitter 1996), since all alien species are expected to lose their natural enemies. Surprisingly little is known about intraspecific PSF in alien non-invasive species as most former studies dealing with the importance of intraspecific PSF for plant invasiveness either compared PSF in invasive and native species, or PSF of a species in their invasive and native range. Only a very few studies compared PSF of invasive and alien non-invasive species (e.g., Kempel et al. 2013; Zuppinger-Dingley et al. 2011) or PSF of alien species in area where they are invasive with areas where they are alien but not invasive (e.g., Andonian et al. 2011). It is not clear whether the alien non-invasive species also exhibit positive intraspecific PSF and their invasion is limited by some other traits, time since introduction or frequency of planting, or if their PSF is negative and prevents them from becoming invasive. Knowledge of the differences in the feedback between invasive and alien non-invasive plants is, however, crucial for assessing the importance of PSF for plant invasiveness and an existence of such difference could help us to predict which alien species have the potential of becoming invasive and which do not.

One of the main goals of my dissertation was to compare intraspecific PSF of a large set of alien species differing in their invasive status. I decided to study only intraspecific feedback as intraspecific feedback can be viewed as a species characteristic that can be compared with other species characteristics. In contrast, interspecific feedbacks are contingent upon the other tested species and it is thus much harder to compare it with other species characteristics. In addition, there are in fact more studies dealing with interspecific feedback in invasive species than studies dealing with intraspecific feedback. Considering intraspecific PSF as species trait also allowed me to assess its relative importance in comparison with a wide range of other plant characteristics used to explain plant invasiveness in previous studies (reviewed e.g. in van Kleunen et al. 2010a). The information on other characteristics was taken from previous studies using the same set of species (Kubesova et al. 2010; Moravcova et al. 2010) and from the LEDA traitbase (Kleyer et al. 2008). Such an analysis provided unique information on the importance of intraspecific plant-soil feedback in comparison with other possible species characteristics explaining plant invasiveness.

Studying PSF of a large set of alien species allowed me to explore other patterns in PSF, for example the relationship of PSF and residence time of the species or their phylogenetic relatedness to native flora. Residence time, i.e. the time since the first introduction of the species to the new area, has been suggested to be negatively correlated with PSF since in longer time it is more likely that local pathogens will colonize or adapt to the focal alien species (Dostal et al. 2013; Flory and Clay 2013). However, the literature has shown mixed support for this idea, with one study showing increasingly more negative intraspecific PSF with residence time of aliens (Diez et al. 2010) and two others showing no effect (McGinn et al. 2018; Speek et al. 2015). One of the reasons for the disparity across studies might be that the studies have not considered the invasive success of the aliens. The expected negative pattern might be particularly pronounced for non-invasive aliens that get regulated by the accumulating pathogens while the invasive species might be less prone to pathogen accumulation and thus be able to attain dominance (Kulmatiski et al. 2008). Moreover, the previous studies have used relatively small sets of species, not allowing for general conclusions to be drawn. A study on a large set of alien species which differ in invasive success and for which the residence time is mostly known (Pysek et al. 2012b) can thus provide an important insight into this issue.

Phylogenetic relatedness of alien species to native flora is also expected to play a role in PSF since closely related plant species are more likely to share pathogens (Bufford et al. 2016; Gilbert and Parker 2016; Parker et al. 2015; Vacher et al. 2010). It is therefore possible that alien species with a close relative in native flora have more negative PSF as specialized pathogens might be present in the introduced range or as it might be easier for the local pathogens to adapt to them. No study has so far focused on the effect of phylogenetic relatedness on intraspecific PSF of aliens, but it has been shown that plants experience more negative interspecific PSF when grown in soil conditioned by a closely related species than by a less related species (Callaway et al. 2013; Dostal and Paleckova 2011; Kempel et al. 2018, but see Kutakova et al. 2018; Mehrabi and Tuck 2015; Munzbergova and Surinova 2015).

Besides comparing PSF of alien species differing in invasive status, I also focused on comparisons of PSF of invasive and native species. Many studies have previously compared PSF of invasive and native species, generally concluding that invasive species experience more positive or less negative PSF than native species (Agrawal et al. 2005; Anacker et al. 2014; Engelkes et al. 2008; Klironomos 2002; MacDougall et al. 2011; Perkins and Nowak 2013; van Grunsven et al. 2007). However, most of the studies have compared PSF of invasive species that are common in the introduced range with PSF of native species that are rare or far less common in the same area. Since PSF is known to be linked to species commonness and rarity (Kempel et al. 2018; Klironomos 2002; MacDougall et al. 2011; van der Putten et al. 2013), it is not clear whether the observed differences in PSF between invasive and native species are more related to the species origin, or to their different abundance in the field. In my study, I therefore compared PSF of invasive and native dominants from the same type of habitat.

A possible limitation of PSF experiments is that they are done only under one type of conditions. Conditions of plant cultivation, both biotic and abiotic, are likely to affect the results of PSF since they affect plants, soil biota or both (De Long et al. 2019; Smith-Ramesh and Reynolds 2017; Whitaker et al. 2017). Not considering multiple conditions of plant cultivation may be the reason why many PSF experiments yield results that are either unpredictable or inconsistent between the glasshouse and the field (Heinze and Joshi 2018; Heinze et al. 2016; Kulmatiski et al. 2008). While the effects of the biotic drivers such as competition or herbivory on the results of PSF gained quite a lot of attention in the past years (e.g., Casper and Castelli 2007; Crawford and Knight 2017; Heinze and Joshi 2018; Hol et al. 2013; Muller et al. 2016; Schittko et al. 2016; Shannon et al. 2012), only a very few studies have so far focused on the role of abiotic conditions of plant cultivation on the results of PSF experiments (e.g., Fry et al. 2018; Kaisermann et al. 2017; Png et al. 2018).

Abiotic conditions of plant cultivation, such as moisture, temperature, shading or nutrient levels, are supposed to have effect on the results of PSF for several reasons. First, they represent an additional form of stress for the plants that can make them more vulnerable to negative soil effects such as soil pathogens (Suzuki et al. 2014). Second, soil microorganisms respond to abiotic conditions such as temperature or moisture and their direct effects on the plants, as well as on nutrient cycling and decomposition rates, thus change accordingly (Heinze et al. 2015; Valliere and Allen 2016; van der Putten et al. 2016). Also, the conditions of plant cultivation alter plant biomass allocation and therefore change the intensity of the interactions between plants and soil organisms (Baxendale et al. 2014; Bergmann et al. 2016; Cortois et al. 2016).

Another possible limitation of PSF studies is that they usually focus only on PSF during the vegetative stages of plant life (Kardol et al. 2013) with a few exceptions targeting germination, survival or establishment of seedlings (Liu et al. 2015; Mangan et al. 2010; Packer and Clay 2000), or production of reproductive biomass as a proxy of plant fitness (Burns et al. 2017). It has been hypothesized by Kardol et al. (2013) and shown by Dudenhoffer et al. (2018) that PSF can change in intensity and even in direction throughout plant's life. PSF in early stages of plant life tends to be more positive than during maturity (Dudenhoffer et al. 2018) since juvenile plants have less developed root system and can thus benefit more from associations with mycorrhizal fungi (Aldrich-Wolfe 2007; van der Heijden 2004). Since different stages of plant life depend on different components of soil biota and different groups of soil biota respond differently to changes in abiotic conditions, it is also possible that the effect of conditions of plant cultivation on the results of PSF differs between life cycle stages.

While studying general characteristics that promote plant invasiveness, I also wanted to understand the biology of individual invasive species. As a model, I chose an invasive species *Impatiens parviflora* and studied not only its PSF, but also focused on factors affecting its spread and assessed its impact on native vegetation. *I. parviflora* is one of the most widely spread invasive species in central Europe (Lambdon et al. 2008), colonizing a wide spectrum of habitats (Pysek et al. 2012a; Sadlo et al. 2007). *I. parviflora* is along with other *Impatiens* species a popular model species for studying plant invasions in central Europe because the genus *Impatiens* contains a species native to central Europe (*I. noli-tangere*), invasive species (*I. parviflora* and *I. glandulifera*), as well as alien species that do not invade despite being planted in the introduced range (*I. balsamina*, *I. scabrida* and *I. balfourii*), allowing for multiple comparisons unbiased by different phylogeny. Despite the large numbers of comparative studies that include *I. parviflora* as one of *Impatiens* species (e.g., Cuda et al. 2014; 2015; 2016; Godefroid and Koedam 2010; Skalova et al. 2012; Skalova et al. 2011), spread dynamics of the species itself, as well as its impact on native vegetation has been surprisingly little monitored. Similarly, no information on its PSF is available, as the only *Impatiens* studied in terms of PSF has been *I. glandulifera* (Pattison et al. 2016).

There are many possible ways how to study factors affecting the spread of invasive species and their ecological requirements. Most of the studies have so far used a space-for-time substitution, i.e. comparing the characteristics of invaded and non-invaded habitat (Pickett 1989). While this approach has many advantages, it is also limited by several problems. For example, using this approach, the possibility that the species simply did not have enough time to enter the habitat is not considered. Similarly, the approach cannot distinguish between the causes and consequences of the invasion as many species modify the environmental characteristics of the invaded habitat (Rejmánek et al. 2013). Last, this approach does not allow for disentangling between individual phases of invasion. Absence of a species in a habitat can be caused by many different factors (e.g., lack of seed availability in the area, seeds not being able to germinate, seedlings not being able to establish, plants not being able to complete their life cycle, plants not producing enough seeds), all of which result in different implications for the management of the invasive species.

One of the methods that allows for determination of factors which affect the spread of an invasive species without the above-mentioned complications is to observe its spread into a new habitat from the very beginning. It can be done either by observing natural spread of the species in early phases of invasion in some area, or by using a sowing experiment. These studies have only rarely been conducted in the past (Becerra and Bustamante 2011; Cheplick 2010; Christen and Matlack 2009; Crosti et al. 2016; Miller and Matlack 2010) as they are much more time-consuming than the space-for-time substitution approach. In addition, in sowing experiments one carries the responsibility for introducing invasive plants into non-invaded habitats, and for observing natural spread, it is not easy to find a suitable area where the species is not yet widely dispersed, but where it is in the infancy of spreading, especially for already established invaders.

Similarly problematic is the methodology of studying the impact of invasive species on native vegetation. Impacts of most invasive plants have never been studied experimentally (Barney et al. 2015; Guido and Pillar 2015), and our knowledge on their impact comes, similarly to studies dealing with factors affecting invasive plants spread, from observational studies that have compared invaded and non-invaded habitats (Levine et al. 2003). As species composition and diversity may themselves influence the likelihood of invasion, separating cause and effect using such correlative approach is nearly impossible (Levine and D'Antonio 1999). It is therefore more appropriate to use experimental approaches such as experimental introduction (e.g., Flory and Clay 2010; Maron and Marler 2008) or removal of the invasive species (e.g., Guido and Pillar 2015; Kumschick et al. 2015). As experimental introductions of invasive species into natural or semi-natural habitats are understandably not encouraged, removal experiments seem to be the best way to study the impact of invasive species on native vegetation (Zavaleta et al. 2001).

In my dissertation, I studied intraspecific PSF of a large set of alien plant species differing in their invasive status and I investigated the relationships between PSF, residence time and phylogenetic novelty of the aliens (Study 1). Next, I assessed differences in PSF of invasive and native dominants (Study 2) and investigated the role of cultivating conditions on results of PSF experiments (Studies 2 and 3). In addition, I studied PSF in *Impatiens parviflora* and compared it to PSF of three alien non-invasive *Impatiens* species (Study 3). Last, I observed natural spread of *I. parviflora* to describe its ecological requirements and dynamics of its spread (Study 4), and I assessed its impact on native vegetation in oak-hornbeam forests using a removal experiment (Study 5).

Overview of Studies

The thesis comprises five studies that are briefly described in this chapter:

Study 1: Aldorfová, A., Knobová, P. & Münzbergová, Z. Plant-soil feedback contributes to predicting plant invasiveness of 68 alien plant species differing in invasive status. *Manuscript*.

Study 2: Aldorfová, A. & Münzbergová. Conditions of plant cultivation affect the differences in intraspecific plant-soil feedback between invasive and native dominants. *Manuscript*.

Study 3: Florianová, A. & Münzbergová, Z. (2018). The intensity of intraspecific plant-soil feedbacks in alien *Impatiens* species depends on the environment. *Perspectives in Plant Ecology, Evolution and Systematics* 32: 56-64.

Study 4: Florianová, A. & Münzbergová, Z. (2018). Drivers of natural spread of invasive *Impatiens parviflora* differ between life-cycle stages. *Biological Invasions*, 20: 2121-2140.

Study 5: Florianová, A. & Münzbergová, Z. (2017). Invasive *Impatiens parviflora* has negative impact on native vegetation in oak-hornbeam forests. *Flora* 226: 10-16.

Study 1

In Study 1, we were studying intraspecific plant-soil feedback (PSF) of 68 invasive and alien non-invasive species. We were looking for differences between PSF of invasive and alien non-invasive species, for the relationship between PSF and time since introduction, and for the relationship between PSF and phylogenetic novelty of the alien species. In this study, we used the same set of species that was previously used for studying genome traits (Kubesova et al. 2010) and reproductive characteristics (Moravcova et al. 2010) of invasive and alien non-invasive neophytes of the Czech Republic. That allowed us to assess the relative importance of PSF for plant invasiveness in comparison with a wide range of other species characteristics, such as plant functional traits, residence time or phylogenetic novelty. We aimed to answer the following questions: i) Do invasive species have less negative (or more positive) intraspecific PSF than alien non-invasive species? ii) Do phylogenetically novel species have less negative (or more positive) intraspecific PSF than species with a close native relative? iii) What is the relationship between residence time of alien species and their intraspecific PSF? Is the relationship the same for invasive and alien non-invasive species? iv) How important is intraspecific PSF for predicting alien plant invasiveness compared to other plant characteristics commonly studied?

Results of this study showed that intraspecific PSF plays a role in plant invasions and that PSF of juvenile plants is more important than PSF of adult plants. Invasive plants had more positive PSF for seedling establishment, but not for biomass, than alien non-invasive plants. PSF for seedling establishment belonged to five best predictors of plant invasiveness, along with specific leaf area, height, seedling growth rate and residence time. These results were consistent across different measures of plant invasion success (invasion status, frequency in the field and maximum cover in natural communities). PSF was related to phylogenetic novelty expressed as absence of a native congener in Czech flora and to residence time of the alien species. Phylogenetically novel species experienced less negative PSF than species with native congeners, suggesting they benefit more from enemy release. PSF of alien non-invasive species was becoming more negative with increasing residence time suggesting accumulation of pathogens, however, PSF of invasive species did not show this pattern.

Study 2

In Study 2, we were comparing intraspecific PSF of invasive and native dominants under different types of cultivating conditions. For this experiment, we chose three pairs of invasive and native congeneric species with similar level of dominance in the Czech Republic, and we assessed PSF of different stages of their life in a standard two-phase feedback experiment. During the first phase, we conditioned the soil by the species, and in the second phase we compared their growth in soil conditioned by the same species and in control, not conditioned soil. To account for the importance of conditions of plant cultivation, we grew the plants in the second phase of the experiment under four different types of conditions – two levels of watering combined with two levels of shading. We aimed to answer the following questions: i) Do invasive species differ from native species with the same level of dominance in terms of intraspecific PSF? ii) Are the results of PSF experiments affected by cultivating conditions? iii) Do cultivating conditions differ in their effect on PSF of invasive vs. native species and different performance measures, such as seedling establishment, aboveground and belowground biomass and root-shoot ratio? iv) Is there any relationship between allocation to belowground biomass and intensity of PSF?

Both invasive and native dominants showed neutral to positive PSF for seedling establishment and negative PSF for biomass. PSF for seedling establishment and aboveground biomass showed no overall difference between invasive and native dominants, but differences existed under some cultivating conditions. PSF for seedling establishment was affected by moisture with the response of individual genera reflecting their ecological requirements. PSF for aboveground biomass was affected by the interaction of moisture and shading and was most negative under the dry light treatment. PSF for aboveground biomass was negatively correlated to root biomass, indicating that higher investment into roots under dry conditions might lead to intensified interactions with soil biota and thus more negative plant-soil feedback.

Study 3

In Study 3, we were studying intraspecific PSF of invasive *Impatiens parviflora* and three other alien non-invasive *Impatiens* species growing in the Czech Republic, under four types of cultivating conditions, similarly to Study 2. We aimed to answer the following questions: i) What is the intraspecific PSF of individual *Impatiens* species?, ii) Does the invasive *I. parviflora* have more positive PSF that the other three *Impatiens* species?, iii) Are the results PSF experiments affected by the cultivating conditions?, iv) Are there any differences in the effect of the cultivating conditions on PSF between species?, v) Are different plant performance measures (seedling establishment, total biomass, root-shoot ratio) affected by the cultivating conditions in the same way?

All studied *Impatiens* species showed positive PSF under some types of cultivating conditions, which indicates they might have a potential to become invasive. PSF for total biomass and for root-shoot ratio were significantly affected by the cultivating conditions. Individual species and studied performance measures responded differently to individual treatments. In most cases, the feedback was changing from positive in optimal treatment to neutral or even negative under some suboptimal treatments. No effect of the cultivating conditions on PSF for seedling establishment was observed. These results, along with results of Study 2, indicate that cultivating conditions play an important role in PSF. We showed that some feedbacks detected in highly controlled experiments using just one type of cultivating conditions might not exist in the field, and similarly some existing feedbacks might remain undetected. We therefore recommend using multiple cultivating conditions or conditions closely resembling conditions in natural sites to increase the realism of the results.

Study 4

In Study 4, we were studying the factors affecting natural spread of *Impatiens parviflora* in different types of plant communities. We found a locality where *I. parviflora* just started spreading, and we established 15 transects starting with a source population and continuing to non-invaded vegetation in six different types of habitats. The transects were divided into 1×1 m plots and we observed the spread and performance of *I. parviflora* in the plots in five consecutive years. In addition, we measured various biotic and abiotic characteristics in each plot and used them for predicting the spread and performance of *I. parviflora*. We aimed to answer the following questions: i) What is the temporal dynamics of *I. parviflora* spread in different habitats? ii) What are the factors affecting different demographic rates (seedling emergence and establishment, survival to maturity, fitness) of *I. parviflora*?

The results showed that individual stages of *I. parviflora* life-cycle were affected by individual environmental conditions to different extents. The most important factor preventing seedling emergence and establishment was a high cover of herb layer, which, however, did not affect survival of older plants. Thus, *I. parviflora* can grow in sites with dense cover of herb layer in case the cover forms after *I. parviflora* seedlings establish. Juvenile mortality was the highest in sites with low nutrient levels and low soil moisture. Canopy openness had a negative effect on *I. parviflora* performance. *I. parviflora* performed better in neutral soils in comparison to acidic soils. Oak-hornbeam forests were the most suitable habitat for *I. parviflora,* followed by acidophilous oak and mixed coniferous forests. However, *I. parviflora* was able to penetrate even into species-rich habitats such as thermophilous oak forests or steppe grasslands on rocks which makes it a potential threat to biodiversity. Only heathlands found on former pastures proved to be unsuitable for *I. parviflora*, as these remained uninvaded until the end of the study.

Study 5

In Study 5, we were studying the impact of *I. parviflora* on native vegetation in oak-hornbeam forests using a removal experiment. We established nine pairs of plots in invaded vegetation, removed all individuals of *I. parviflora* from one plot of each pair, and observed the changes in species composition in the plots for four years. To reveal any general characteristics of the species suppressed by *I. parviflora* invasion, we correlated the species response to *I. parviflora* removal with the species traits. We aimed to answer the following questions: i) What is the effect of *I. parviflora* removal on species composition, number and cover of native species? ii) What are the traits of the species most suppressed by *I. parviflora* invasion?

The results show that *I. parviflora* has negative effect on native vegetation since both numbers and cover of native species were increasing in the removal plots in comparison with the invaded plots. The greatest change occurred in the first two years after the invader removal, indicating the vegetation recovers relatively quickly. Changes in species composition were also observed – species that were increasing in cover in the removal plots, i.e. species most restricted by *I. parviflora* invasion, were mostly species with small releasing height and early start of flowering.

Summary

In the presented studies, we showed that PSF matters in plant invasions. PSF has been shown by previous studies to allow invasive plants to gain dominance over native species (e.g., Kulmatiski et al. 2008; Meisner et al. 2014; van Grunsven et al. 2007), and to be positively linked with plant abundance in the field (Klironomos 2002; Mangan et al. 2010, but see Maron et al. 2016; Reinhart 2012, for no or opposite effect). Here, in Study 1, we showed that PSF also differentiates invasive from less successful alien species, and that it is actually a better predictor of invasive success of aliens than many other species characteristics commonly considered, such as size or shape of propagules, genome size or ploidy level. There are many possible mechanisms that could explain why alien species differ in direction and intensity of their intraspecific PSF. Successful alien plant species may be less regulated by enemies and/or benefit more from the interactions with soil mutualists (Fitter 2005; Menzel et al. 2017; Reinhart and Callaway 2006), or they may have an intrinsic potential to improve the soil environment to their favor, for example via affecting decomposition rates and nutrient cycling (Ehrenfeld 2004; Hu et al. 2018; Wolfe and Klironomos 2005).

Some of our results indicate that the degree of enemy release plays a role in differentiating PSF of alien plants. For example, we showed that phylogenetically novel species experience more positive PSF than species with close native relatives, suggesting that they are released from natural enemies to greater extent (Bufford et al. 2016; Gilbert and Parker 2016; Parker et al. 2015; Vacher et al. 2010). Similarly, differentiated relationship between the residence time and PSF for invasive and alien non-invasive plants suggests that individual alien plants are either released from enemies to different extent (MacLeod et al. 2010) or that they differ in the rate of subsequent accumulation or adaptation of local pathogens (Dickie et al. 2017). However, since all species included in the study are alien species and thus are at least to some extent released from natural enemies, the results suggest that there also must be intrinsic characteristics of species that determine the strength and direction of their PSF.

There have been attempts to determine which plant traits affect strength and direction of PSF. Commonly studied plant traits, such as plant relative growth rate, plant life-span, growth form, plant size or specific leaf area, have been shown to affect PSF, though their predictive power is generally relatively low (Orwin et al. 2010; Kulmatiski et al. 2008; Baxendale et al. 2014; Meisner et al. 2014; Kulmatiski et al. 2017; Kuťáková et al. 2018). Traits that are expected to be more closely connected with PSF, such as content of various nutrients in the plant tissues and various root traits, have been shown to affect PSF by some studies, but not others (Lemmermeyer et al. 2015; Cortois et al. 2016; Bukowski et al. 2018; Bennett and Klironomos 2018; Semchenko et al. 2018). Production of root exudates, that are generally recognized as important determinants of plant interactions with its environment as they are directly affecting soil chemistry, soil biota as well as the growth of other plant species (Meier et al. 2017; Hu et al. 2018), has not yet received explicit attention in PSF studies. In addition, toxicity of self-DNA (i.e. DNA of the same species) may be another important, yet overlooked, mechanism of intraspecific PSF (Mazzoleni et al. 2015).

The existing studies on the trait-PSF relationships are usually limited by use of too few species or too few simple-to-measure plant traits and are thus not able to provide more comprehensive understanding of the trait-PSF relationship. We would thus like to use the PSF data collected in Study 1 in a future study to explore the relationship between PSF and plant traits. As plant traits, we would use detailed chemical analyses (content of phosphorus, nitrogen, carbon, calcium, magnesium, lignins and phenols) of plant above- and below-ground biomass that was stored from the experiment, as well as root morphology, production of root exudates and self-DNA content that would be measured in newly planted individuals. This study would provide mechanistic insights into the determinants of intraspecific PSF and would thus allow us to further understand which plant traits affect PSF and consequently plant invasiveness as well.

In Study 2, we showed that invasive plants do not differ from native plants in terms of net PSF when both plant species reach similar level of dominance in the field. However, we do not know what the processes behind the feedbacks are. The mechanisms of establishing the PSF may originate from different aspects of plant-soil interaction for invasive and native species. The overall plant performance results from a combination of antagonistic (e.g., pathogens) and mutualistic (e.g., mycorrhiza) interactions, as well as physical and chemical properties of the soil (Reinhart and Callaway 2006; van der Putten et al. 2013). While the invasive species might benefit from enemy release, the native species might benefit from better adaptations to local mutualists, resulting in comparable net PSF. Disentangling the relative contributions of different antagonistic and mutualistic soil organisms would thus provide important insights into the underlying mechanisms of the feedbacks. In a concurrent project comprising two diploma theses in progress, we thus study PSF of invasive and native dominants in more details. First, we analyze both biotic and abiotic composition of the soil after the conditioning phase, second, we compare growth of the species in 12 treatments, which allows us to not only assess the changes in microbial communities, but also to quantify their effect on the plant performance.

The treatments include six treatments with conditioned and six treatments with not conditioned soil – non-sterilized, sterilized, sterilized inoculated with complete inoculum from conditioned or from not conditioned soil, and sterilized inoculated with bacterial filtrate of conditioned or not conditioned soil.

All our PSF experiments (Studies 1, 2 and 3) showed that PSF for seedling establishment significantly differs from PSF for adult plants, and that PSF of individual life stages responds differently to the cultivating conditions, suggesting that individual components of PSF are of different importance for juvenile and for adult plants (Kardol et al. 2013). PSF for seedling establishment generally tends to be more positive than PSF for adult plants, probably because seedlings have less developed root system and can thus benefit more from associations with mycorrhizal fungi (Aldrich-Wolfe 2007; van der Heijden 2004). Importantly, PSF for seedling establishment was a better predictor of plant invasiveness than PSF for biomass of adult plants in our study, indicating that early stages of plant lives are crucial for plant invasions, as suggested by previous studies as well (Gioria and Pysek 2017; Grotkopp and Rejmánek 2007; Skalova et al. 2012; Van Kleunen and Johnson 2007). Similarly, we showed in Study 4 that factors controlling germination and seedling emergence of *I. parviflora* differ from those controlling survival or fitness of adult plants. Our findings are in line with previous studies that have detected contrasting effects of soil on different stages of plant life (e.g., Brandt et al. 2013; Deck et al. 2013; Dudenhoffer et al. 2018; Munzbergova and Surinova 2015), as well as studies from other fields of plant ecology demonstrating different effects of various ecological factors on individual stages of plant life (e.g., Dostalek and Munzbergova 2013; Knappova et al. 2013; Munzbergova et al. 2013). These results point to the neccessity of considering multiple stages of plant life cycle in PSF experiments (Dudenhoffer et al. 2018; Kardol et al. 2013) as well as in studies on dispersal and establishment of alien plants.

Two of the studies presented in this thesis (Studies 2 and 3) pointed to the importance of cultivating conditions, namely moisture and shading levels, on results of PSF experiments. We showed interactive effects of moisture and shading on PSF of focal species, with PSF being most negative under the conditions least suitable for growth of given species. This shows that plants are more vulnerable to negative soil effects when simultaneously stressed by the environmental conditions (Suzuki et al. 2014). PSF was generally most negative under the dry light treatment which can be explained by altered biomass allocation. We showed that PSF for aboveground biomass was negatively correlated with root mass. Under the dry light treatment, plants increased allocation to root biomass and thus probably interacted more intensively with soil pathogens, as suggested by previous studies (Bergmann et al. 2016; Cortois et al. 2016; Smith and Reynolds 2015). Importantly, PSF of native and invasive species differed in their response to cultivating conditions, as did PSF of individual stages of plant development of a species. These results demonstrate that the effect of cultivating conditions on PSF is largely context dependent, hardly predictable, changes with other environmental variables and depends on whether the PSF is driven by abiotic factors, pathogens or mutualists. It is therefore important to either include multiple cultivating conditions in future PSF experiments or use conditions as close to natural conditions as possible to increase the realism of the results.

In Studies 3, 4 and 5, I focused in more detail on biology of *I. parviflora* and other *Impatiens* species. In a PSF experiment of Study 3, both *I. parviflora* and three alien non-invasive *Impatiens* species showed positive intraspecific PSF at least under some types of cultivating conditions. For *I. parviflora*, this result was expected as it is an invasive species being dominant in many habitats in the Czech Republic (Pysek et al. 2012a; Sadlo et al. 2007) and as positive PSF has been also reported for the other invasive *Impatiens* in central Europe, *I. glandulifera* (Pattison et al. 2016). Positive PSF of the non-invasive species indicate they might have the potential to become invasive as well. Alternatively, they may differ in other characteristics from the invasive species and their invasion may be limited by those. For example, they might be not so well adapted to local climate or they might differ from the invasive species in plant traits important for *Impatiens* invasion, such as seed mass, time to seed germination, seedling growth rate or biomass production (Cuda et al. 2016). It is also important to note that all the studied non-invasive species were introduced to the Czech Republic later than *I. parviflora* (Pysek et al. 2012b) and are not frequently cultivated (Cuda et al. 2016). As naturalization success and invasive spread are strongly affected by both time since introduction (Pysek and Jarosik 2005) and propagule pressure (Colautti et al. 2007; Cuda et al. 2016), it is possible that these species possess an intrinsic invasiveness that, due to differences in invasion history, may not have reached its full potential yet.

Observing natural spread of *I. parviflora* into new habitats (Study 4) illustrated that monitoring natural spread of invasive species can provide important insights into the determinants of their distribution. It also illustrated that the determinants depend on spatial scale studied and differ between different life stages of the species. Using a removal experiment (Study 5), we showed that *I. parviflora* has negative impact on native vegetation of oak-hornbeam forests, but that the vegetation recovers quite quickly after the invader removal. Due to its extensive distribution and high turnover, removal of the species from larger areas is not realistic in practice. However, as we showed that *I. parviflora* can invade also species-rich habitats often hosting endangered species, we recommend performing a similar experiment in these habitats, monitoring *I. parviflora* spread through them and consider its removal in case of a more intense invasion.

To conclude, we showed that PSF is one of the mechanisms distinguishing between invasive and alien non-invasive plants and that it is of similar importance as other plant characteristics commonly considered in assessing plant invasiveness. We showed that PSF for seedling establishment is a better predictor of plant invasiveness than PSF based on biomass of adult plants, pointing to the importance of early phases of life cycle for invasions and highlighting the need to include multiple stages of plant life cycle in future PSF experiments. Last, we pointed to the importance of cultivating conditions for the results of PSF experiments and we recommend using multiple environmental conditions or conditions closely resembling those found in natural sites in future PSF experiments to increase the realism of the results.

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Study 1

Plant-soil feedback contributes to predicting plant invasiveness of 68 alien plant species differing in invasive status

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(Manuscript)

Abstract

Understanding what species characteristics allow some alien plants to become invasive while others fail to is critical to our understanding of community assembly processes. While many characteristics have been shown to predict plant invasiveness, the importance of plant-soil feedback (PSF) in invasions has been difficult to assess since individual studies include only a few species and use disparate methodology.

We studied PSF of 68 invasive and non-invasive alien species in a single two-phase common garden experiment, and compared the relative importance of PSF, residence time, phylogenetic novelty and plant traits for plant invasiveness. Additionally, we tested for relationships between PSF, residence time and phylogenetic novelty.

PSF for seedling establishment belonged to five best predictors of plant invasiveness, along with specific leaf area, height, seedling growth rate, and residence time. Invasive species had more positive PSF for seedling establishment, but not for biomass, than non-invasive species. Phylogenetically novel species experienced less negative PSF than species with native congeners, suggesting they benefit more from enemy release. PSF of non-invasive species, contrary to that of invasive species, was becoming more negative with increasing residence time.

We demonstrated that PSF for seedling establishment plays a role in predicting invasiveness that is comparable with other species characteristics that are more commonly studied. It should thus receive more attention in future PSF studies.

Key words: alien / exotic / non-native species; enemy release hypothesis; intraspecific (conspecific) plant-soil feedback; invasive ecology; neophytes; plant–soil (below-ground) interactions; phylogenetic relatedness; residence time; specific leaf area.

Introduction

Understanding the causes of biological invasions is a priority of ecological research in the last decades (Sol et al., 2012). Invasive plants displace native species, change vegetation structure, reduce native biodiversity (Hejda et al., 2009, Powell et al., 2013), undermine 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 plant invasiveness and to prevent the emergence of new invasive species.

Many previous studies have examined species characteristics that promote plant invasiveness and differentiate invasive species from their non-invasive relatives. Common characteristics that have been considered, and showed importance in some systems, are plant traits (Rejmanek and Richardson, 1996, van Kleunen et al., 2010), phylogenetic novelty (Strauss et al., 2006), residence time (Pysek and Jarosik, 2005) or plant-soil feedback (Callaway et al., 2004, Kulmatiski et al., 2008, Klironomos, 2002). However, studies only rarely compare the predictive power of multiple species characteristics (Lowry et al., 2013), which allows their relative importance to be ranked, and none of the comparisons have so far included information on plant-soil feedback.

Intraspecific plant-soil feedback (PSF) has been suggested to play an important role in plant invasions. Invasive species generate more positive or less negative intraspecific PSF than native species (e.g., van Grunsven et al., 2007, Kulmatiski et al., 2008, Klironomos, 2002, Meisner et al., 2014). Invasive species also often experience less negative intraspecific PSF in their introduced range compared to the native range due to enemy release (e.g., Reinhart and Callaway, 2004, Callaway et al., 2004, Maron et al., 2014, Yang et al., 2013, Gundale et al., 2014, Reinhart et al., 2010, Halbritter et al., 2012). The extent to which PSF may predict plant invasiveness, i.e. determine the invasion success of alien plants, however, remains unclear. Alien species differ in the degree of enemy release as well as in the way they interact with individual components of soil biota. Some alien species are therefore likely to develop less negative or more positive PSF than other alien species, and thus are more likely to become invasive. A synthesis on this topic is lacking due to limited number of alien non-invasive species that have been studied and due to the disparate methodology across studies. A meta-analysis of Kulmatiski et al. (2008) suggested that invasive species create less negative PSF than non-invasive alien species. Their analysis was, however, based on only a few non-invasive aliens studied in independent experiments and all occurring in grassland ecosystems. The results thus need to be confirmed in a single study with more species, consistent methodology and with species from other ecosystems.

The strength of intraspecific PSF is also expected to be related to other species characteristics than invasive status, such as residence time and phylogenetic novelty of the focal alien. PSF might become more negative with increasing residence time as local pathogens colonize or adapt to the focal alien (Dostal et al., 2013, Flory and Clay, 2013). The literature has shown mixed support for this idea, with one study showing increasingly more negative intraspecific PSF with residence time of aliens (Diez et al., 2010) and two others showing no effect (Speek et al., 2015, McGinn et al., 2018). One of the reasons for the disparity across studies might be that the studies did not consider the invasive success of the aliens. The expected negative pattern might be particularly pronounced for non-invasive aliens that get regulated by the accumulating pathogens while the invasive species might be less prone to pathogen accumulation and thus be able to attain dominance (Kulmatiski et al., 2008).

PSF should be more negative for aliens that are closely related to plants in the native flora because related plant species are more likely to share pathogens (Bufford et al., 2016, Vacher et al., 2010, Parker et al., 2015, Gilbert and Parker, 2016). While it has been shown that PSF is phylogenetically conserved, i.e. that close relatives exhibit more similar feedback responses than phylogenetically distant species (Anacker et al., 2014), no studies examined the effect of phylogenetic novelty of aliens on their intraspecific PSF. However, research on interspecific PSF (experiments that compare plant fitness in soil conditioned by the focal plant and soil conditioned by another plant species) demonstrated that plants experience more negative interspecific PSF when grown in soil conditioned by a congener than in soil conditioned by a less related species (Callaway et al., 2013, Dostal and Paleckova, 2011, Kempel et al., 2018, but see Kutakova et al., 2018, Munzbergova and Surinova, 2015, Mehrabi and Tuck, 2015).

In this study, we quantified intraspecific PSF of 68 alien species of the Czech Republic that vary in their invasive status, abundance and dominance in the field. We evaluated the direct and interactive effects of invasive status, residence time and phylogenetic novelty on PSF. Moreover, we compiled data on a broad range of plant traits (seven whole plant traits, five regenerative traits and one leaf trait) from trait databases, previous studies using the same set of species (Kubesova et al., 2010, Moravcova et al., 2010) and our own data collection, and ranked the plant traits, PSF, residence time and phylogenetic novelty according to their importance for predicting invasiveness of our focal species. The hypotheses we tested were as follows: i) Invasive species have less negative or more positive intraspecific PSF than non-invasive alien species; ii) Phylogenetically novel species have less negative or more positive PSF than species with a close native relative; iii) PSF is becoming more negative with increasing residence time and the effect is more pronounced for non-invasive species than for invasive species; iv) PSF is as important for predicting plant invasiveness as some other commonly used traits.

Materials and methods

Studied species

In this study, we used 68 neophytes (alien species introduced after 1500 A.D. sensu Pysek et al., 2004) occurring in the Czech Republic (Tables S1-S2). The species were selected based on a list of 93 species used in previous studies of Moravcova et al. (2010) and Kubesova et al. (2010) who compared reproductive characteristics and genome size of alien invasive and non-invasive species, respectively. The original set of species was reduced because i) some species from the list were reclassified to archeophytes by Pysek et al. (2012) and ii) we did not manage to collect seeds or cultivate some of the species. Choosing species from that list allowed us to determine the relative importance of PSF and many other traits studied previously for plant invasiveness.

Invasion status (casual, naturalized non-invasive or invasive, sensu Pysek et al., 2004) of the studied species in the Czech Republic was taken from Pysek et al. (2012). Invasive species in our study are understood as species that form self-replacing populations over many life cycles, produce reproductive offspring, often in very large numbers, at considerable distances from the parent and/or site of introduction, and have the potential to spread over long distances (Pysek et al., 2004). Of the 68 species, 27 neophytes are considered invasive in the Czech Republic and 41 non-invasive. The vast majority of the non-invasive species are classified as naturalized non-invasive (i.e. forming self-sustaining populations, not depending on human intervention, but not spreading (Pysek et al., 2004)) and only three (*Ambrosia trifida, Rudbeckia hirta* and *Sedum rupestre*) as casual (i.e. not forming self-sustaining populations, depending on repeated introductions of propagules (Pysek et al., 2004)). We therefore did not distinguish between non-invasive naturalized and casual species in the analyses and merged them all into one category of alien non-invasive species. For each species, we also recorded their frequency (i.e. number of occupied grid cells) and maximum cover of the species in the field (taken from the Pladias database).

The 68 species analyzed are a highly representative sample, making up 17 % of the total number of 408 naturalized non-invasive neophytes and 44 % of the total number of 61 invasive neophytes of the Czech Republic (Pysek et al., 2012) of the Czech Republic. The species studied belong to 52 genera and 27 families according to the Angiosperm Phylogeny Group classification (Stevens, 2001 onwards) with Asteraceae most represented. Annuals, monocarpic perennials and polycarpic perennials are all well represented among the species, with no difference between invasive and non-invasive species (Table S1). Invasive species do not differ from the non-invasive species in terms of minimum residence time in the Czech Republic (Table S1). The invasive species have significantly higher maximum cover in the field than the non-invasive species and occupy more grid cells (Table S1). Phylogenetic novelty in terms of presence of a native congener in the Czech Republic does not differ between invasive and non-invasive species (Table S1).

Seed collection

Seeds of all species were collected in 2014-2016 in the field from multiple localities (range 1-5, mean 2.5; Table S2) in the Czech Republic, minimum 5 km apart from each other, to account for possible differences between populations. For some species, we used seeds provided by a local commercial supplier (Planta Naturalis Ltd., Markvartice, Czech Republic) as one population. The seeds from each population were used separately in the experiments. In each population, we collected mixture of mature seeds from at least 10 individuals. Seeds were stored in paper bags under room temperature until used. The seeds were always used in the experiment in the year after their collection. The seeds of some species were cold-wet stratified for two months prior sowing (information on stratification requirements of individual species provided by L. Moravcová) and all seeds were surface sterilized with 10% H_2O_2 to reduce the chance of contamination via seed surface fungi prior sowing.

Experimental design

Following commonly used methodology (Bever et al., 1997, Kulmatiski et al., 2008), the plants were grown in a two-phase experiment. In the first (conditioning) phase, conditioned soil was prepared. In the second (feedback) phase, intraspecific PSF was studied. More details on individual phases follow in subsequent sections. The experiment was carried out in the experimental garden of Institute of Botany, Czech Academy of Sciences (49°59′38.972″N, 14°33′57.637″E), 320 m above sea level, temperate climate zone, where the mean annual temperature is 8.6°C and the annual precipitation is 610 mm. In addition to obtaining natural rainfall, the plants were daily watered with tap water. Due to high number of studied species, the experiment was divided into three subsequent years, 2015-2017. Each year, we grew approximately the same proportion of invasive and non-invasive species.

Conditioning phase

The aim of the conditioning phase was to prepare the soil, conditioned by the species, for the upcoming feedback phase. To set up the conditioning phase, we used a local common garden soil mixed with sand in 1:1 ratio. We decided not to use soil from the field as common in other PSF studies (Smith-Ramesh and Reynolds, 2017) because we used a high number of species that do not co-occur in nature and collecting soil from a variety of sites would have introduced additional variation due to soil type, fertility, pH etc. The soil collected within our experimental garden was a good compromise to fulfill ecological requirements of all the selected species. The garden is situated in the region where the studied species occur, the local soil was previously conditioned by a mixture of species growing in the region and thus contains the soil biota commonly encountered by the species. The soil was nutrient rich ensuring that the PSF effects will not be driven by nutrient depletion of the soil (see Table S3 for the chemical characteristics of the soil). We prepared a homogenous heap of substrate at the beginning of the first year of the project and stored it in a dry place. To minimize possible soil heterogeneity due to different processes in the different parts of the stored soil, the soil was thoroughly mixed every time before use.

For each species and each population, we used 20 pots (10 × 10 × 10 cm) in the conditioning phase, half of which was sown with seeds of the species and the other half of the pots served as controls. We decided to use unconditioned soil exposed to the same conditions as control, as was done in a range of previous studies (e.g., Kardol et al., 2007, Kulmatiski et al., 2011, Perkins and Nowak, 2013, Wang et al., 2013). Alternatively, we considered a more common method of using soil conditioned by the whole community as a control. Selecting such community was, however, not straightforward, as each of the model species is occurring in different habitat and optimal controls would thus differ between species. Importantly, response of each species to its soil was compared to its response to the same control, and we were primarily interested in difference in this log response ratio between different species. Thus, the exact identity of the control does not change the results as long as the controls are comparable among species. Each pot with conditioned soil was randomly assigned its control pot. The pairs of pots were always kept in close proximity to each other throughout the experiment so that they were exposed to the same conditions.

Each of the ten pots per species and population was sown with ten seeds of the species. After the seeds germinated and seedlings established, we counted the seedlings and removed all but the largest one from the pot. Seedlings emerging afterwards were counted and removed. Both pots with and without plants were kept under the same conditions and regularly watered. The soil was conditioned for 12 weeks, similarly to a range of previous studies (e.g., van Grunsven et al., 2007, Meijer et al., 2011, van de Voorde et al., 2011, van Grunsven et al., 2010, Chiuffo et al., 2015, Florianova and Munzbergova, 2018). After the 12 weeks, the plants were harvested, and roots were carefully removed from the soil.

Feedback phase

Ten seeds of a species were sown into each previously conditioned pot as well as to the control pots. For each species and each population, we thus had 10 pots with conditioned and 10 pots with control soil with sown seeds. We did not mix the soil from all the pots conditioned by the same species and population between the conditioning and feedback phase to avoid pseudoreplication (Brinkman et al., 2010, Smith-Ramesh and Reynolds, 2017).

After the seeds germinated and seedlings established, we counted the seedlings and removed all but the largest one from the pot. Seedlings emerging afterwards were counted and removed. Twelve weeks after seed germination, the plants were harvested, divided into aboveground and belowground biomass, dried to a constant weight and weighed. All plants of the same species and population were harvested from all the pots simultaneously.

Other species characteristics affecting plant invasiveness

We compiled data on multiple species characteristics that are often related to plant invasiveness for our species from previously published papers and databases. These included minimum residence time (MRT), phylogenetic novelty, and plant traits. MRT as a number of years elapsed since the first record of occurrence in the Czech Republic was taken from Pysek et al. (2012). As a measure of phylogenetic novelty, we recorded whether or not there is any species of the same genus for each focal species that is native to the Czech Republic using Danihelka et al. (2012). Plant traits considered in this study were germination, seedling growth rate, seedling establishment, propagule weight, propagule length-width ratio, buoyancy and anemochory measured as terminal velocity, taken from Moravcova et al. (2010), ploidy level and genome size, taken from Kubesova et al. (2010), and life history and releasing height, taken from the LEDA traitbase (Kleyer et al., 2008). Additionally, we measured specific leaf area (SLA) for all the species. To do this, we collected 10 leaves without leaf stalks from at least three different individuals per species prior to the harvest of the conditioning phase, dried them, weighed them and estimated the leaf area using the ImageJ program. SLA was then calculated as area/mass and an average value from the 10 measurements was used in further analyses.

Statistical analyses

First, we tested for the effect of soil treatment (i.e. conditioned or control soil) on seedling establishment (here used as number of established seedlings divided by number of sown seeds, i.e. by 10) and on square-root transformed total biomass (sum of aboveground and belowground biomasses) in the feedback phase for each species separately. We used generalized mixed effect models with binomial error distribution for seedling establishment and linear mixed effect models for total biomass, using the R-package 'lmerTest' (Kuznetsova et al., 2017), with population, pairs of pots and year of planting as random effects. These tests provide information on existence of non-neutral intraspecific PSF in each species. For each species, we then calculated PSF index for seedling establishment and total biomass as ln(x/s) where x is performance of each individual plant when grown in the conditioned soil and s is performance of a plant grown in the paired control pot, as suggested by Brinkman et al. (2010). PSF index values for all studied species are presented in Table S2. An index value of less than zero indicates a negative feedback, meaning the plant performs worse in the conditioned soil than in the control soil, while a value greater than zero indicates a positive feedback, meaning the plant performs better in the conditioned soil than in the control soil.

The data from the feedback phase were further analyzed for all the species combined using generalized linear mixed effect models with binomial error distribution for seedling establishment and using linear mixed effect models for square root transformed biomass from the feedback phase as dependent variables. Species, population, pairs of pots, and year of planting were used as random effects, and soil treatment, invasive status, MRT and their interactions as explanatory variables. The same tests were performed with square-root transformed species frequency (frequency in quadrants of the basic grid mapping cells, taken from Pladias) and species cover in the field (maximum cover in the field, taken from Pladias) as alternative measures of plant invasiveness. Since maximum cover had very uneven distribution, we divided the plants into three categories that were approximately equally represented in the dataset [low (less than 10 %), medium (less than 50 %) and high cover (more than 50 %)], and used maximum cover as a discrete variable. The results of these analyses were consistent with those using invasive status and are presented in the Supporting Information (Tables S4-S5, Figs S1-S3). Similarly, we performed tests with phylogenetic novelty (existence of any native species of the same genus in the Czech Republic) instead of MRT. Measures of plant invasiveness are used as explanatory variables in these tests, though biologically invasiveness is a function of PSF and not the other way around. Despite this, we used measures of plant invasiveness as the explanatory variables because such tests allowed us to study the interactions between a measure of invasiveness and soil conditioning, MRT and phylogenetic novelty. It is, however, important to keep this in mind when interpreting the results.

To test for the relative importance of PSF for plant invasiveness in comparison with other species characteristics, we first calculated an average value of PSF index for seedling establishment and for biomass for each species across all populations. Then we used generalized linear models with binomial error distribution with invasive status as a dependent variable and a species characteristic affecting plant invasiveness as an explanatory variable. We performed the model separately for each studied species characteristic and compared delta AIC of all models with a null model. Again, we repeated the analyses with species frequency (linear models on square-root transformed data) and species cover (linear model with multinomial error distribution ('multinom' function in 'nnet' package in R (Venables and Ripley, 2002)), and present these results in Supplementary Information (Table S5).

All analyses were performed twice, once with phylogenetic correction and once without it. To account for phylogenetic signal in the data, we used a method of phylogenetic eigenvector regression (Diniz et al., 1998). First, we extracted a phylogenetic tree for the 68 species in our study from Daphne (Durka and Michalski, 2012). Second, the phylogenetic distance matrix was decomposed into its eigenvectors using PCoA in the R-package 'ape' (Paradis et al., 2004). The first three eigenvectors, that together explained more than 50 % of the variability in the data, were included as covariables in the analyses in order to correct for phylogenetic autocorrelation. Since the analyses with and without the phylogenetic correction produced very similar results, we present only the results of the phylogenetically informed analyses. All analyses were performed using R 2.13.2 (R Development Core Team, 2014).

Results

Significant positive PSF for seedling establishment was shown in four invasive and eight non-invasive species, significant negative PSF in two invasive and six non-invasive species. Significant positive PSF for biomass was shown in three invasive and five non-invasive species, significant negative PFS for three invasive and twelve non-invasive species (Table S2).

Overall, seedling establishment was significantly related to the soil treatment with plants performing better in the cultivated soil compared to the control soil (F = 11.9, P = 0.001). There was a significant interaction of soil treatment and invasive status (F = 3.53, P = 0.05), with seedlings of invasive species profiting more from growing in conditioned soil, i.e. having more positive PSF, than seedlings of non-invasive species (Fig. 1). Biomass was significantly affected by the soil treatment (F = 24.5, P < 0.001) with plants growing worse in the conditioned soil compared to the control soil, i.e. showing negative PSF. There was no significant difference in the response of invasive and non-invasive species to the soil conditioning for plant biomass.

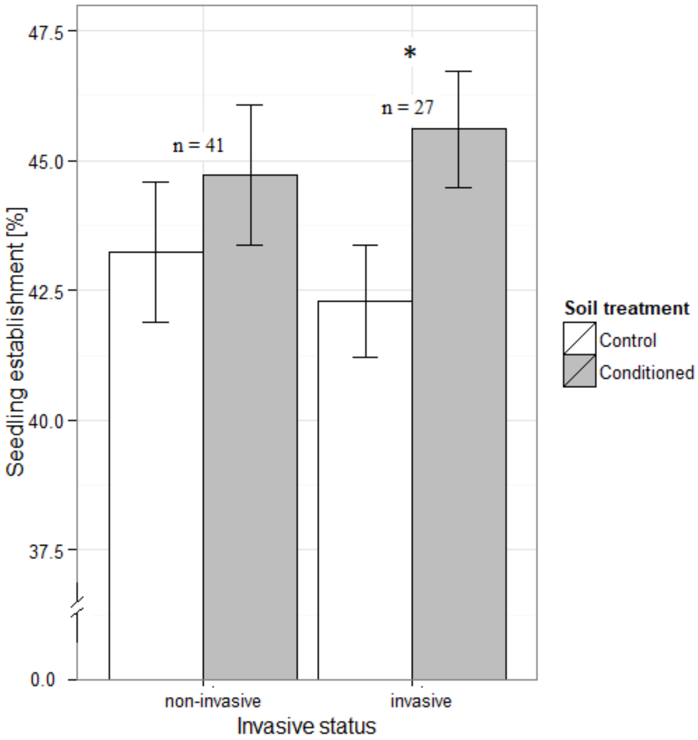
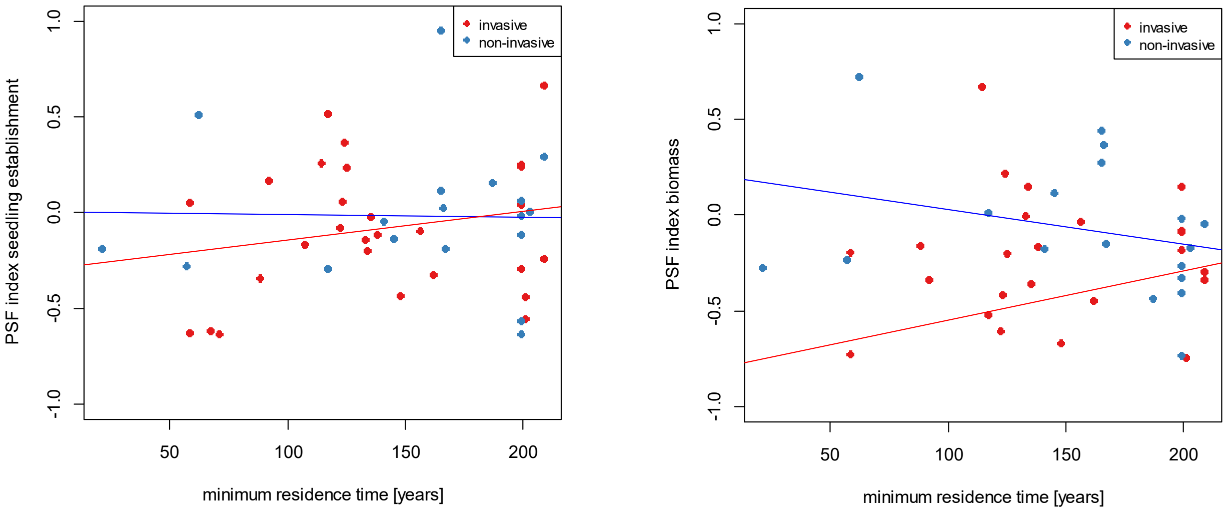


Fig. 1: Seedling establishment of invasive and non-invasive species in control and conditioned soil (mean ± SE). Asterisks indicate significant (P < 0.05) difference between control and conditioned soil. Better performance in conditioned soil compared to control indicates positive PSF, better performance in control soil compared to conditioned soil indicates negative PSF. Number of species in each category in indicated by n.

Neither seedling establishment nor biomass was significantly related to the interaction of MRT and soil treatment when all species were considered. However, triple interactions of MRT, soil treatment and invasion status were significant (F = 20.92, P < 0.001 for seedling establishment, F = 5.57, P = 0.018 for biomass). We therefore tested for the effect of MRT also separately for invasive and non-invasive species and found opposite relationships in the two categories (Fig. 2). While PSF of invasive species was positively correlated to MRT (F = 16.3, p < 0.001 for seedling establishment, Fig. 2a, F = 3.14, P = 0.076 for biomass, Fig. 2b), PSF of non-invasive species was negatively correlated to MRT (F = 4.76, P = 0.029 for seedling establishment, Fig. 2a, F = 7.28, P = 0.007 for biomass, Fig. 2b).



(a) (b)

Fig. 2: Dependence of PSF index for (a) seedling establishment and (b) biomass on minimum residence time for non-invasive and invasive plant species. Each data point represents mean PSF index of a single species.

Seedling establishment, but not biomass, was significantly affected by the interaction of the soil treatment and phylogenetic novelty of the species (F = 8.29, P = 0.004), while the interaction of phylogenetic novelty with invasive status and the triple interaction between the soil treatment, phylogenetic novelty and invasive status were not significant. Phylogenetically novel species, i.e. species that do not have a congener native to the country, had significantly better seedling establishment in the conditioned soil compared to the control soil, while species that do have a native congener performed comparably in the conditioned and the control soil (Fig. 3).

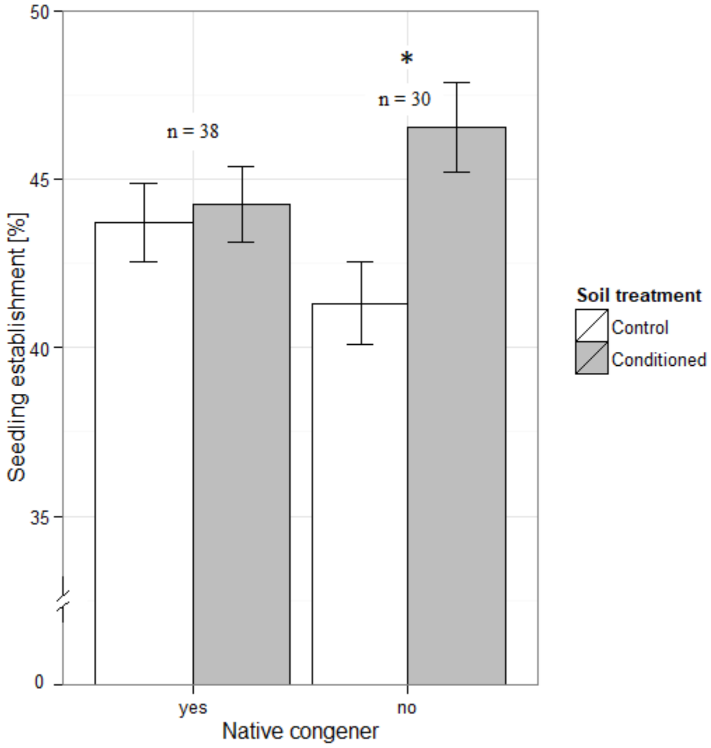


Fig. 3: Seedling establishment of species that do and do not have a native congener in the Czech Republic in control and conditioned soil. Asterisks indicate significant ($P < 0.05$) difference between control and conditioned soil. Better performance in conditioned soil compared to control indicates positive PSF, better performance in control soil compared to conditioned soil indicates negative PSF.

The best predictors of invasive status were SLA, releasing height, MRT, seedling growth rate and PSF index for seedling establishment (Fig. 4). Similar results were obtained when predicting species frequency and maximum cover in the field (Table S5).

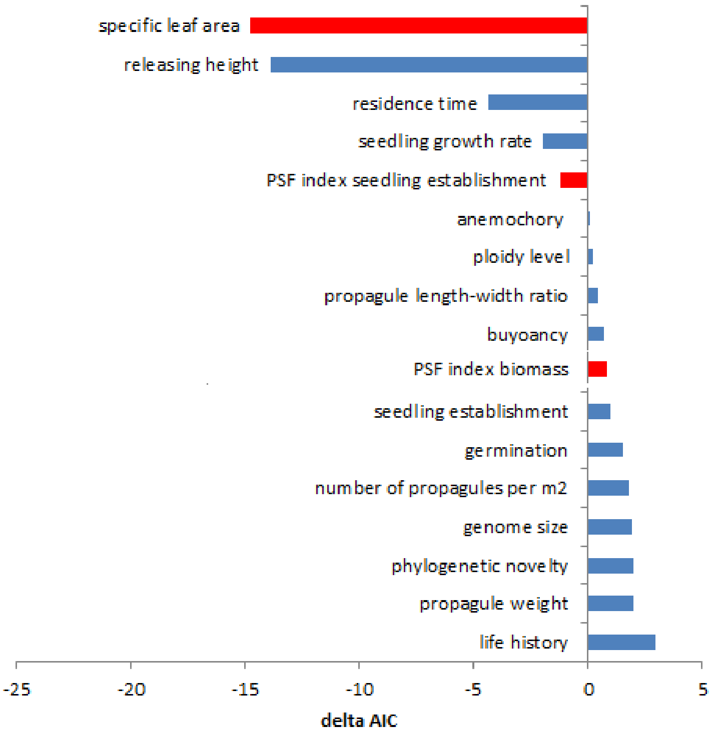


Fig. 4: Delta AIC for models studying the effect of various species characteristics on invasive status. Negative values indicate significant contribution to explaining invasiveness. Red bars represent characteristics measured directly in our study, blue bars characteristics taken from other studies (see Methods).

Discussion

In the present study, we demonstrated on a large set of species that invasive species have more positive PSF for seedling establishment, but not for biomass, than alien non-invasive species. We showed that PSF for seedling establishment belonged to the best predictors of plant invasiveness compared to a wide range of other species characteristics, even though its explanatory power was rather low. We also showed that PSF is affected by phylogenetical novelty of the aliens and that it depends on residence time of the species.

Our study considered 68 alien plant species and 17 species characteristics, including PSF, providing a unique analysis of the relative importance of different predictors of plant invasiveness. The five most important predictors were specific leaf area, height, seedling growth rate, MRT and PSF for seedling establishment. We found that tall plants with high SLA and fast seedling growth rates are more likely to become invasive, which corresponds to previous studies (Pyšek and Richardson, 2007, van Kleunen et al., 2010, Ordonez et al., 2010). High specific leaf area is correlated with fast growth rate (Grotkopp et al., 2002) and height is positively associated with long-distance dispersal ability and with ability to compete for light (Thomson et al., 2011). Together, these traits allow plants to better disperse to and succeed in the disturbed habitats. We found that alien plants with longer residence time are more likely to be classified as invasive. This is also in line with other research (Pysek and Jarosik, 2005, Pysek et al., 2009), and our research highlights the importance of MRT in relation to many other invasion mechanisms, as has been previously done for woody plants (Pysek et al., 2009). Importantly, alien species are more likely to be classified as invasive if they have more positive PSF in their seedling establishment. PSF responses are not currently considered in programs that evaluate alien plants for invasiveness, and seedling establishment is not a commonly measured response variable in PSF research. Our results show that PSF especially for seedling establishment should receive more attention in future studies since PSF was a better predictor of invasiveness than many other species characteristics commonly considered, such as size or shape of propagules, genome size or ploidy level.

We showed that invasive species have more positive PSF for seedling establishment than non-invasive aliens, regardless of whether invasive success was categorical or based on maximum cover or frequency in the field. PSF has been shown to allow invasive plants to gain dominance over native species in many previous studies (e.g., Kulmatiski et al., 2008, Meisner et al., 2014, van Grunsven et al., 2007), and to be positively linked with plant abundance in the field (Klironomos, 2002, Mangan et al., 2010, but see Reinhart, 2012, Maron et al., 2016, for no or opposite effect). Our study shows that PSF also differentiates invasive from less successful alien species, even though the differences were not too prominent. There are many possible mechanisms that could explain this result. Successful alien plant species may be less regulated by enemies and/or benefit more from the interactions with soil mutualists (Fitter, 2005, Reinhart and Callaway, 2006, Menzel et al., 2017). In addition, successful aliens may have an intrinsic potential to improve the soil environment to their favor, for example via affecting decomposition rates and nutrient cycling (Ehrenfeld, 2004, Wolfe and Klironomos, 2005, Hu et al., 2018).

PSF for seedling establishment played a more important role for plant invasiveness than PSF for biomass in our study system, confirming that early stages of plant lives are crucial for successful invasion (Gioria and Pysek, 2017). This is an important finding because seedling establishment is not commonly considered in PSF research (Kardol et al., 2013) and we know little about how plant-soil interactions affect seedling recruitment. A few studies have shown that PSF of seedlings may largely differ from that of adults and tends to be more positive

(Dudenhoffer et al., 2018, Florianova and Munzbergova, 2018). This may be because seedlings have a less developed root system and can thus benefit more from associations with mycorrhizal fungi (Aldrich-Wolfe 2007; van der Heijden 2004). On the other hand, seedlings may be very vulnerable to negative effects of soil pathogens in some systems (Packer and Clay, 2000, Liu et al., 2015). The number of established seedlings in our study could have also been affected by different germination rates that might be subject to PSF as well. Some plants release specific root exudates that stimulate plant growth-promoting rhizobacteria (van Loon, 2007, Vacheron et al., 2013, Hu et al., 2018), and there is some evidence, mostly from agricultural systems, that these can positively affect seed germination (e.g., Kloepper et al., 1991, Wu et al., 2016). Some plants have also been shown to release chemicals that directly stimulate germination of seeds. This has mostly been observed in interspecific interactions and the focal seeds are typically of parasitic plants (Netzly et al., 1988, Hameed et al., 1973, Fernandez-Aparicio et al., 2009, Fernandez-Aparicio et al., 2008). However, it is possible that similar mechanisms are involved in intraspecific PSF.

PSF of aliens is expected to become more negative over time due to adaptation and accumulation of pathogens (Dostal et al., 2013, Flory and Clay, 2013), but this pattern has received mixed support (Diez et al., 2010, Speek et al., 2015, McGinn et al., 2018). Our study suggests that this inconsistency could be due to the lack of explicit consideration of invasive status. In our study, non-invasive aliens showed the expected negative pattern with MRT, while invasive aliens showed an opposite relationship. Most studies examining the release from belowground enemies have focused on problematic invaders and little is known about the release of non-invasive aliens (Callaway et al., 2004, Reinhart et al., 2003, van Grunsven et al., 2007, Gundale et al., 2014, Maron et al., 2014, Yang et al., 2013). Alien plants might differ in the number of enemy taxa that initially accompany them to the new regions (MacLeod et al., 2010) and in the rate at which they subsequently accumulate enemies (Dickie et al., 2017). However, plant-soil mutualist interactions may also accumulate over time (Dickie et al., 2017) and the balance between accumulation of pathogens and mutualists will determine the net PSF pattern. It has been suggested that mutualist limitation, even though not so common and often temporary, may reduce the rate of population growth and abundance and prevent a naturalized species from becoming invasive (Dickie et al., 2017). Moreover, it has been documented that problematic invasive plants may benefit from novel soil mutualists (Reinhart and Callaway, 2004, Reinhart and Callaway, 2006, Sun and He, 2010), and more studies are needed to determine if such patterns differ between invasive and non-invasive aliens.

Alien species that are phylogenetically related to the native flora should be more likely to share species-specific pathogens (Bufford et al., 2016, Vacher et al., 2010, Parker et al., 2015, Gilbert and Parker, 2016). They should therefore develop more negative or less positive PSF than phylogenetically novel species that are only attacked by generalist pathogens, having usually considerably smaller effect on plants than specialists (Colautti et al., 2004). On the contrary, soil mutualists, such as AM fungi, whose accumulation in the soil might lead to positive PSF, usually have low level of endemism (Davison et al., 2015) and lower host specificity (Smith and Read, 2008, Molina and Horton, 2015). The alien species can thus usually benefit from their presence regardless of their phylogenetic relatedness to native flora (Yang et al., 2014, Richardson et al., 2000, McGinn et al., 2016). Previous studies have demonstrated that plants grow poorly when grown in soil conditioned by a close relative (Callaway et al., 2013, Dostal and Paleckova, 2011, Kempel et al., 2018, but see Kutakova et al., 2018, Munzbergova and Surinova, 2015, Mehrabi and Tuck, 2015). However, our study aimed to examine whether or not novelty of aliens can explain PSF patterns across many alien plant species, all growing in resident soil. Indeed, we found the expected pattern that alien species with a native congener in the Czech Republic have less positive PSF than phylogenetically novel species. Our results together with other literature show that the presence of closely related native plant species has negative effects on alien species via the activity of herbivores, phytophagous insects, aboveground or belowground pathogens (e.g., Connor et al., 1980, Flory and Clay, 2013, Hill and Kotanen, 2009, Parker et al., 2015).

The majority of species in our study showed neutral PSF, which contradicts the general worldwide pattern that most species show negative intraspecific PSF (e.g., Kulmatiski et al., 2008, Petermann et al., 2008). This is likely to be caused by our study focusing only on aliens which may be at least partly released from negative effects of their host-specific enemies (see also the neutral and positive PSF of invasives reported by Callaway et al., 2004, Reinhart and Callaway, 2006, Meisner et al., 2014, Suding et al., 2013, Engelkes et al., 2008, Levine et al., 2006). In addition, the strength of PSF might depend on duration of soil conditioning or type of soil used (van de Voorde et al., 2012, Lepinay et al., 2018). In our study, we used local common garden soil mixed with sand while the majority of previous studies used soil collected directly in the localities. Our soil could thus initially contain lower abundances of species specific pathogens, which would cause slower pathogen accumulation during the conditioning phase causing experimental results to be less negative. The soil also contained more nutrients reducing the likelihood of negative PSF due to nutrient depletion. Using soil from the field was not possible in our experiment as the species do not co-exist in the field and collecting soil from a variety of sites would have introduced additional variation due to soil type, fertility, pH etc. As the initial soil used for the experiment likely has significant effects on the results of PSF (van de Voorde et al., 2012, Png et al., 2018, Gundale et al., 2019), it would be better to include more different types of soil for each species in our experiment. Doing this would, however, substantially increase the magnitude of the already extensive experiment and would make it hardly feasible to conduct without decreasing the number of studied species. In addition, other previous studies demonstrated that the effects of specific soil used are much weaker than the effects of soil cultivation and do not interact (Hemrova et al., 2016).

Our results have implications for future studies in other regions, for future research on PSF, and possibly for biosecurity screening of alien plants. Our research can serve as a guide for distributed observations and experiments in other regions of the world, although slight modifications of the PSF experiment design should be considered (mainly type of soil and character of control). By considering multiple species characteristics that might explain invasiveness and using consistent methodology across all aliens in the PSF experimentation, our methods allow the importance of different species characteristics to be ranked and the magnitude and direction of PSF to be compared across species. If such an approach was replicated in other regions, this would enable more sophisticated meta-analyses of invasion mechanisms. Our research highlights the need for future work on PSF, particularly focusing on the mechanisms by which these feedbacks influence seedling establishment. Finally, biosecurity screening processes for importing new aliens, could consider evaluating aliens for positive PSF in case future studies in other regions confirm our results.

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Supporting Information

Table S1: Summary characteristics of the neophytes in the Czech flora studied in the present paper, shown separately for invasive (n = 27) and non-invasive (n = 41) alien species. Differences between invasive and non-invasive species tested with chi-square test or one-way ANOVA, for minimum residence time and species frequency on square-root and ln transformed data, respectively.

	Non-invasive	Invasive	Difference
Life history	annuals 15 (37 %) monocarpic perennials 9 (22 %) polycarpic perennials 17 (41 %)	annuals 13 (48 %) monocarpic perennials 4 (15 %) polycarpic perennials 10 (37 %)	$X^2 = 1.378$; df = 2; P = 0.502
Minimum residence time (years, mean ± sd)	144.7 ± 54.6	156.5 ± 37.6	F = 1.289; df = 1, 59; P = 0.261
Max cover (%, mean ± sd) (min, max)	35.6 ± 31.4 (1-88)	70.4 ± 35.2 (1-99)	F = 5.1427; df = 1, 55; P = 0.027
Species frequency (number of occupied grids, mean ± sd); (min, max)	392.3 ± 534.8 (5-1851)	657.3 ± 563.5 (30-2190)	F = 7.722; df = 1, 65; P = 0.007
Phylogenetic novelty (absence of native congener)	Yes – 17 (41 %) No – 24 (59 %)	Yes – 13 (48 %) No – 14 (52 %)	$X^2 = 0.591$; df = 1; P = 0.442

Table S2: List of studied species, their description and PSF indices for biomass and seedling establishment. P-values indicate whether seedling establishment or biomass in the feedback phase are affected by soil treatment (conditioned, not-conditioned). Significant values (P < 0.05) are in bold, marginally significant values (P < 0.1) in italics. Life history: an – annual, mono – monocarpic perennial, per – polycarpic perennial. Invasion status: inv – invasive, non-inv – non-invasive. Minimum residence time – number of years elapsed since the first record of occurrence in the Czech Republic. Species frequency – number of colonized quadrants of basic cells in grid mapping. Maximum cover – maximum cover in the field. Number of populations – number of populations studied. NA – information not available.

Species	Family	Life history	Invasion status	Minimum residence time [years]	Species frequency	Maximum cover [%]	Seedling establishment		Biomass		Number of populations
							PSF index (mean ± sd)	p-value	PSF index (mean ± sd)	p-value	
Abutilon theophrasti Med.	Malvaceae	an	non-inv	124	61	4	**0.372 ± 0.247**	**0.014**	0.018 ± 0.537	0.649	3
Amaranthus albus L.	Amaranthaceae	an	non-inv	125	181	63	0.24 ± 0.523	0.251	-0.331 ± 0.817	0.485	3
Amaranthus powellii S. Watson	Amaranthaceae	an	inv	165	433	88	*0.06 ± 0.627*	*0.063*	-0.075 ± 0.47	0.866	2
Amaranthus retroflexus L.	Amaranthaceae	an	inv	200	931	63	**0.686 ± 0.881**	**0.001**	-0.093 ± 0.763	0.168	3
Ambrosia artemisiifolia L.	Asteraceae	an	inv	135	92	1	-0.022 ± 0.268	0.588	0.07 ± 1.263	0.761	4
Ambrosia trifida L.	Asteraceae	an	non-inv	58	8	88	0.055 ± 0.58	0.111	**-0.183 ± 0.337**	**0.013**	3
Antirrhinum majus L.	Plantaginaceae	mono	non-inv	199	70	3	**0.245 ± 0.648**	**0.034**	-0.019 ± 0.613	0.512	3
Asclepias syriaca L.	Apocynaceae	per	inv	117	86	88	*0.161 ± 0.55*	*0.075*	**0.407 ± 0.497**	**0.008**	3
Aster lanceolatus Willd.	Asteraceae	per	inv	NA	189	NA	**-0.747 ± 0.358**	**<0.001**	**0.439 ± 0.464**	**0.005**	3
Bidens frondosus L.	Asteraceae	an	inv	124	1360	88	-0.012 ± 0.57	0.836	0.162 ± 0.343	0.393	2
Bunias orientalis L.	Brassicaceae	mono	inv	162	344	88	*-0.324 ± 0.5*	*0.053*	*-0.441 ± 0.675*	*0.059*	2
Cannabis ruderalis Janisch.	Cannabinaceae	an	inv	150	30	3	0.01 ± 0.294	0.298	0.157 ± 0.861	0.304	2
Cardamine chelidonia L.	Brassicaceae	mono	non-inv	88	20	NA	-0.34 ± 0.538	0.2	-0.063 ± 0.739	0.509	2
Chenopodium pumilio R. Br.	Amaranthaceae	an	non-inv	128	113	3	**-0.442 ± 0.365**	**0.015**	**-1.719 ± 1.449**	**0.001**	1
Chenopodium strictum Roth	Amaranthaceae	an	non-inv	NA	532	2	**-0.611 ± 0.697**	**0.047**	**-0.321 ± 0.646**	**0.014**	5
Claytonia alsinoides Sims	Montiaceae	an	non-inv	67	NA	NA	**-0.618 ± 0.585**	**0.003**	**-1.772 ± 1.243**	**<0.001**	2
Collomia grandiflora Lindl.	Polemoniaceae	an	non-inv	138	5	NA	-0.111 ± 0.583	0.902	-0.135 ± 0.514	0.268	3
Conyza canadensis (L.) Cronq.	Asteraceae	an	inv	268	1463	88	**0.957 ± 1.005**	**0.025**	0.475 ± 0.656	0.133	3
Datura stramonium L.	Solaginaceae	an	non-inv	209	286	38	**0.586 ± 1.013**	**<0.001**	0.342 ± 1.913	0.683	4
Digitalis purpurea L.	Plantaginaceae	mono	non-inv	228	650	63	-0.375 ± 0.858	0.879	-0.079 ± 0.733	0.279	2
Duchesnea indica (Andrew) Focke	Rosaceae	per	non-inv	58	38	1	**-0.626 ± 0.501**	**0.009**	**-0.593 ± 1.26**	**0.015**	2

							Seedling establishment		Biomass		
Species	Family	Life history	Invasion status	Minimum residence time [years]	Species frequency	Maximum cover [%]	PSF index (mean ± sd)	p-value	PSF index (mean ± sd)	p-value	Number of populations
Echinocystis lobata (Michx.) Torr. et Gray	Cucurbitaceae	an	inv	107	253	88	-0.166 ± 0.366	0.803	**-1.26 ± 0.82**	**0.027**	1
Echinops sphaerocephalus L.	Asteraceae	per	inv	147	729	88	-0.156 ± 0.45	0.727	0.009 ± 0.289	0.684	2
Epilobium ciliatum Rafin.	Onagraceae	per	inv	92	1851	38	**0.168 ± 0.784**	**0.011**	-0.176 ± 1.454	0.831	4
Erigeron annuus (L.) Pers.	Asteraceae	mono	inv	134	388	63	0.110 ± 0.171	0.341	0.414 ± 0.607	0.104	1
Galinsoga parviflora Cav.	Asteraceae	an	inv	138	1198	88	*-0.17 ± 0.303*	*0.1*	0.248 ± 1.07	0.314	4
Galinsoga quadriradiata Ruiz et Pavón	Asteraceae	an	inv	117	1215	88	**0.52 ± 0.436**	**0.007**	-0.113 ± 1.244	0.591	1
Geraniaceaenium pyrenaicum Burm. fil.	Geraniaceae	per	non-inv	199	530	38	0.125 ± 0.39	0.139	-0.101 ± 0.285	0.52	3
Helianthus tuberosus L.	Asteraceae	per	inv	133	578	99	-0.139 ± 0.619	0.859	0.081 ± 1.602	0.177	2
Heracleum mantegazzianum Sommier et Levier	Apiaceae	mono	inv	156	694	99	-0.097 ± 0.18	0.203	-0.028 ± 0.526	0.565	2
Hesperis matronalis L.	Brassicaceae	per	non-inv	201	552	18	**-0.482 ± 0.502**	**0.007**	**-0.469 ± 0.612**	**0.012**	2
Impatiens glandulifera Royle	Balsaminaceae	an	inv	122	1214	90	-0.079 ± 0.494	0.92	**-0.471 ± 0.633**	**0.005**	3
Impatiens parviflora DC.	Balsaminaceae	an	inv	148	2190	99	-0.434 ± 0.335	0.645	*-0.442 ± 0.642*	*0.093*	2
Imperatoria ostruthium L.	Apiaceae	per	non-inv	209	123	63	*-0.239 ± 0.299*	*0.077*	0.068 ± 1.102	0.863	3
Iva xanthiifolia Nutt.	Asteraceae	an	non-inv	71	55	88	-0.637 ± 0.891	0.35	**-1.331 ± 1.168**	**0.017**	1
Kochia scoparia (L.) Schrader	Amaranthaceae	an	inv	199	138	88	-0.288 ± 0.55	0.133	-0.046 ± 0.279	0.435	3
Lepidium densiflorum Schrader	Brassicaceae	mono	non-inv	114	244	38	**0.259 ± 0.281**	**0.046**	**0.747 ± 0.645**	**0.008**	1
Lupinus polyphyllus Lindl.	Fabaceae	per	inv	123	1167	70	-0.092 ± 0.374	0.682	-0.142 ± 0.572	0.692	3
Lychnis coronaria (L.) Desr.	Caryophyllaceae	mono	non-inv	139	67	2	-0.521 ± 0.636	0.731	-0.454 ± 0.469	**0.015**	3
Lysimachia punctata. L.	Primulaceae	per	non-inv	199	423	2	*0.254 ± 0.575*	*0.073*	0.283 ± 0.875	0.294	2
Matricaria discoidea DC.	Asteraceae	an	non-inv	165	1733	88	**0.954 ± 0.548**	**<0.001**	0.393 ± 1.459	0.101	2
Medicago sativa L.	Fabaceae	per	non-inv	199	1020	63	*-0.113 ± 0.403*	*0.078*	-0.183 ± 0.485	0.246	2
Mimulus guttatus DC.	Phrymaceae	per	non-inv	165	163	13	0.096 ± 0.719	0.104	**0.33 ± 0.58**	**<0.001**	4
Oenothera biennis L.	Onagraceae	mono	non-inv	187	621	NA	*-0.132 ± 0.501*	*0.078*	-0.237 ± 0.511	0.114	3
Oenothera glazioviana M. Micheli	Onagraceae	mono	non-inv	128	177	NA	0.183 ± 0.282	0.183	**-0.146 ± 0.291**	**0.017**	2
Oxalis dillenii Jacq.	Oxalidaceae	mono	inv	NA	75	2	0.201 ± 0.454	0.404	**0.463 ± 0.966**	**0.018**	3
Oxalis fontana Bunge	Oxalidaceae	mono	non-inv	166	1043	38	0.024 ± 0.535	0.458	**0.643 ± 1.127**	**0.048**	1
Phytolacca esculenta Van Houtte	Phytolaccaceae	per	non-inv	62	35	NA	**0.51 ± 0.871**	**<0.001**	**0.916 ± 2.127**	**<0.001**	3
Rudbeckia hirta L.	Asteraceae	per	non-inv	145	77	38	0.068 ± 0.556	0.208	0.193 ± 0.556	0.317	2
Rudbeckia laciniata L.	Asteraceae	per	inv	159	343	13	0.217 ± 0.477	0.118	-0.013 ± 0.983	0.846	1
Rumex alpinus L.	Polygonaceae	per	non-inv	199	65	90	-0.026 ± 0.305	0.865	**-0.405 ± 0.429**	**<0.001**	3
Rumex longifolius DC.	Polygonaceae	per	non-inv	NA	46	NA	0.064 ± 0.38	0.423	-0.203 ± 0.623	0.178	2
Rumex patientia L. subsp. patientia	Polygonaceae	per	inv	157	32	38	-0.111 ± 0.352	0.777	-0.172 ± 0.442	0.402	2
Rumex thyrsiflorus Fingerh.	Polygonaceae	per	non-inv	NA	401	13	**-0.765 ± 0.76**	**0.031**	*-0.206 ± 0.526*	*0.079*	3

Species	Family	Life history	Invasion status	Minimum residence time [years]	Species frequency	Maximum cover [%]	Seedling establishment		Biomass		Number of populations
							PSF index (mean ± sd)	p-value	PSF index (mean ± sd)	p-value	
Scutellaria altissima L.	Lamiaceae	per	non-inv	117	18	NA	-0.29 ± 0.521	0.395	0.037 ± 0.218	0.705	1
Sedum hispanicum L.	Crassulaceae	per	non-inv	NA	334	2	**0.441 ± 0.573**	**0.003**	-0.573 ± 1.357	0.278	3
Sedum rupestre L. subsp. erectum t´Hart	Crassulaceae	per	non-inv	NA	61	38	0.091 ± 0.474	0.342	**-1.779 ± 1.147**	**<0.001**	1
Senecio inaequidens DC.	Asteraceae	per	non-inv	21	116	NA	-0.186 ± 0.801	0.939	0.009 ± 1.281	0.512	3
Setaria faberi F. Herrmann	Poaceae	an	non-inv	57	19	1	-0.28 ± 0.608	0.4	*-0.211 ± 0.321*	*0.07*	1
Sisymbrium altissimum L.	Brassicaceae	an	non-inv	203	287	88	0.008 ± 0.588	0.9	**0.633 ± 1.72**	**0.034**	4
Sisymbrium loeselii L.	Brassicaceae	an	inv	199	409	88	**-0.668 ± 1.005**	**0.002**	**-0.37 ± 0.996**	**0.009**	3
Sisymbrium strictissimum L.	Brassicaceae	per	non-inv	199	325	13	-1.226 ± 0.661	0.133	**-0.584 ± 1.654**	**<0.001**	1
Solidago canadensis	Asteraceae	per	inv	180	1202	99	1.644 ± 0.423	0.389	0.097 ± 0.498	0.392	1
Telekia speciosa (Schreber) Baumg.	Asteraceae	per	inv	198	305	1	-0.074 ± 0.659	0.813	-0.066 ± 0.889	0.776	3
Trifolium hybridum L.	Fabaceae	mono	non-inv	199	1783	88	0.031 ± 0.497	0.353	*0.361 ± 1.342*	*0.093*	4
Veronica persica Poiret	Plantaginaceae	an	non-inv	209	1838	63	**0.296 ± 0.471**	**0.002**	-0.018 ± 0.348	0.707	3
Vicia grandiflora Scop.	Fabaceae	an	non-inv	141	42	NA	-0.041 ± 0.325	0.922	*-0.120 ± 0.367*	*0.085*	3
Xanthium albinum (Widd.) H. Scholtz et Sukopp	Asteraceae	an	non-inv	167	106	2	-0.142 ± 0.303	0.443	-0.048 ± 0.372	0.593	3

Table S3: Abiotic characteristics of the soil prior to the conditioning phase. Values show the mean and stardard deviation of six samples. The analyses were performed by the Analytical Laboratory of Institute of Botany, Czech Academy of Sciences, Průhonice. The methods used for the analyses are described in detail in Raabova et al. (2008).

	mean ± sd
pH(H_20)	7.77 ± 0.03
pH(KCl)	7.70 ± 0.01
total N [%]	0.07 ± 0.02
total C [%]	1.01 ± 0.20
exchangeable Ca [mg/kg]	1259.35 ± 125.63
exchangeable Mg [mg/kg]	105.03 ± 11.53
exchangeable K [mg/kg]	278.60 ± 21.61
exchangeable P [mg/kg]	39.99 ± 1.12
total P [mg/kg]	159.46 ± 46.70

Table S4: Results of mixed effect models with i) seedling establishment and ii) biomass from the feedback phase as dependent variables and phylogenetic eigenvectors (axes 1-3), MRT, soil treatment, measure of invasion and their interaction as explanatory variables. Measure of invasion: invasion status, square-root transformed species frequency and maximum cover, respectively. Species, populations, pairs of pots, and year of planting were used as random effects. DenDF - Satterthwaite approximation for degrees of freedom. NumDF = 1 for all variables. Significant values ($P < 0.05$) are in bold, marginally significant values ($P < 0.1$) are in italics. Species frequency – number of colonized quadrants of basic cells in grid mapping. Maximum cover – based on maximum cover in the field, taken from Pladias, transformed into a discrete variable with three levels (low cover < 10 %, medium 11-50 %, high > 50 %).

			axis1	axis2	axis3	MRT	soil treatment	measure of invasion	meas. of invasion * soil treat.
Seedling establishment	invasion status	DenDF	161.3	*160.2*	160.4	**161.0**	**3037.1**	160.6	*3037.0*
		F	1.54	*3.37*	0.00	**8.26**	**11.90**	0.05	*3.53*
		P	0.216	*0.068*	0.973	**0.005**	**0.001**	0.823	*0.050*
	species frequency	DenDF	159.4	*158.2*	158.6	**158.6**	2996.4	**330.0**	**2999.4**
		F	1.73	*3.36*	0.07	**8.34**	1.27	**5.36**	**9.88**
		P	0.190	*0.069*	0.259	**0.004**	0.259	**0.005**	**0.002**
	maximum cover	DenDF	134.3	**133.2**	133.5	**133.9**	**2546.7**	*133.6*	**2546.4**
		F	0.00	**6.92**	0.11	**4.71**	**10.78**	*2.74*	**5.61**
		P	0.965	**0.010**	0.739	**0.032**	**0.001**	*0.069*	**0.004**
Biomass	invasion status	DenDF	161.3	160.7	*160.8*	161.1	**3026.7**	160.9	3026.5
		F	1.45	0.01	*2.98*	1.83	**24.50**	0.47	1.50
		P	0.230	0.920	*0.086*	0.178	**<0.001**	0.494	0.220
	species frequency	DenDF	159.3	158.7	158.9	158.9	**2987.2**	312.5	2988.6
		F	1.42	0.26	1.05	0.85	**10.45**	1.17	0.17
		P	0.236	0.611	0.306	0.359	**0.001**	0.217	0.679
	maximum cover	DenDF	134.2	133.7	133.8	134.1	**2545.5**	133.9	*2545.4*
		F	0.80	0.61	0.06	0.03	**14.23**	0.93	*2.54*
		P	0.372	0.438	0.814	0.860	**<0.001**	0.396	*0.079*

Table S5: Comparison of delta AIC for models studying the effect of various species characteristics on invasive status, species frequency and maximum cover in the field. Underlined variables are data obtained in this study. For invasive status, we used generalized linear models with binomial error distribution, for species frequency linear models on square-root transformed data and for maximum cover linear models with multinomial error distribution (‘multinom’ function in ‘nnet’ package in R (Venables and Ripley, 2002)).

	invasion status		species frequency		maximum cover	
	delta AIC	rank	delta AIC	rank	delta AIC	rank
specific leaf area	**-23.217**	**1**	**-170.788**	**1**	**-41.780**	**1**
releasing height	**-21.801**	**2**	**-92.106**	**2**	**-18.587**	**2**
MRT	**-6.814**	**3**	**-57.521**	**3**	**-11.483**	**3**
seedling growth rate	**-3.111**	**4**	**-26.486**	**4**	**-1.396**	**7**
PSF seedling establishment	**-1.871**	**5**	**-16.757**	**5**	**-0.024**	**11**
anemochory	0.065	6	**-14.767**	**6**	**-2.460**	**6**
ploidy level	0.227	7	**-6.385**	**8**	**-0.249**	**10**
propagule length-width ratio	0.404	8	**-8.217**	**7**	**-0.608**	**8**
buyoancy	0.667	9	1.606	15	3.392	15
PSF biomass	0.814	10	0.997	10	3.508	16
seedling establishment	0.956	11	1.302	14	2.256	13
germination	1.519	12	1.284	13	2.685	14
number of propagules per m^2	1.783	13	1.781	16	3.959	17
genome size	1.932	14	1.113	11	**-4.354**	**5**
native congener	1.982	15	**-3.193**	**9**	0.2077	12
propagule weight	1.991	16	1.245	12	**-0.354**	**9**
life history	2.979	17	3.012	17	**-4.447**	**4**

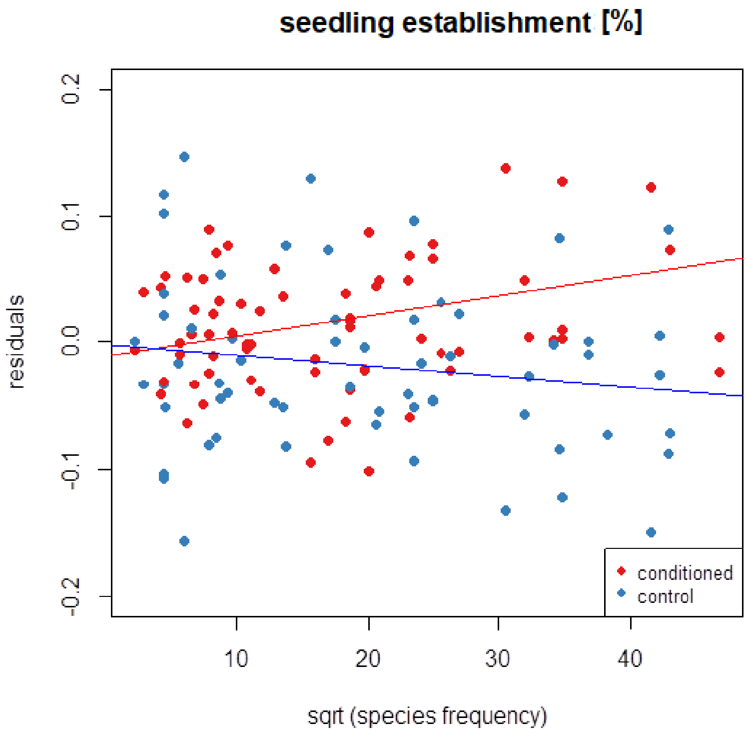

Fig. S1: Dependence of mean residuals for seedling establishment across species, after accounting for phylogenetic information, MRT and random effect of species, population, year of planting and pairs of pots, on square-root transformed species frequency in quadrants of the basic grid mapping cells for conditioned and control soil. Better performance in conditioned soil compared to control indicates positive PSF, better performance in control soil compared to conditioned soil indicates negative PSF.

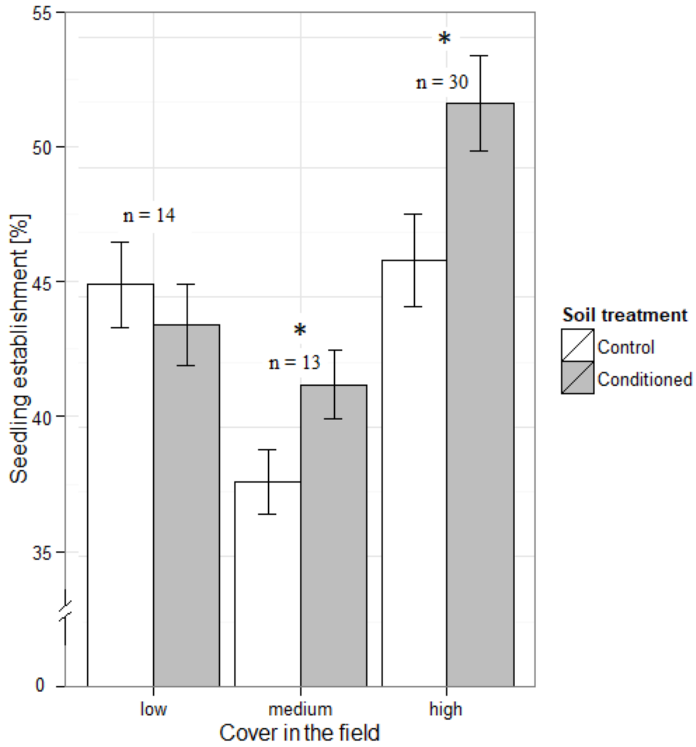

Fig. S2: Seedling establishment of species with low (< 10 %), medium (11-50 %) and high (> 50 %) maximum cover in the field in control and conditioned soil (mean ± SE). Asterisks indicate significant ($P < 0.05$) difference between control and conditioned soil. Better performance in conditioned soil compared to control indicates positive PSF, better performance in control soil compared to conditioned soil indicates negative PSF. Number of species in each category in indicated by n.

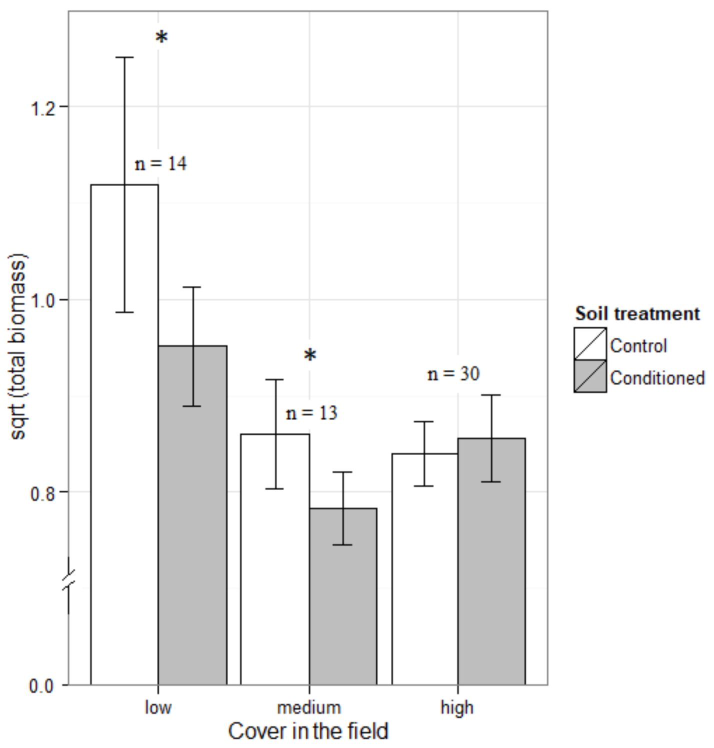

Fig. S3: Square-root transformed biomass of species with low (< 10 %), medium (11-50 %) and high (> 50 %) maximum cover in the field in control and conditioned soil (mean ± SE). Asterisks indicate significant ($P < 0.05$) difference between control and conditioned soil. Better performance in conditioned soil compared to control indicates positive PSF, better performance in control soil compared to conditioned soil indicates negative PSF. Number of species in each category in indicated by n.

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Study 2

Conditions of plant cultivation affect the differences in intraspecific plant-soil feedback between invasive and native dominants

Anna Aldorfová and Zuzana Münzbergová

(Manuscript)

Abstract

Intraspecific plant-soil feedback (PSF), a process in which plants affect the soil and the modified soil affects further growth of the same plant species, has been proposed to be one of the mechanisms controlling plant dominance in the field as well as plant invasiveness. However, very few studies compared the PSF of invasive species with native species of a similar level of dominance. In this study, we compared PSF of three pairs of invasive and native congeners with the same level of dominance in the field and with similar ecological requirements. Additionally, we assessed the role of conditions of plant cultivation for the PSF by growing the plants under four treatments (two moisture × two shading regimes).

Both invasive and native dominants showed neutral to positive PSF for seedling establishment and negative PSF for biomass. Native species had more negative PSF for belowground biomass than invasive species. PSF for seedling establishment and aboveground biomass showed no overall difference between invasive and native dominants, although differences existed under some cultivating conditions. PSF for seedling establishment was affected by moisture with the response of individual genera reflecting their ecological requirements. PSF for aboveground biomass was affected by the interaction of moisture and shading and was most negative under the dry light treatment. PSF for aboveground biomass was negatively correlated to root biomass, indicating that higher investment into roots under dry conditions might lead to intensified interactions with soil biota and thus more negative plant-soil feedback.

Key words: alien species; biomass allocation; drought; enemy release hypothesis; plant-soil interactions; stress.

Introduction

Intraspecific plant-soil feedback (PSF), i.e. the mechanism in which a plant affects both biotic and abiotic characteristics of the soil and the soil then affects further growth of the same species (Bever et al., 1997), has been repeatedly suggested to play an important role in plant invasions (Kulmatiski et al., 2008; Levine and D'Antonio, 1999; Meisner et al., 2014). According to enemy release hypothesis (Elton, 1958; Keane and Crawley, 2002), invasive plants are supposed to escape their natural enemies when moving to a new range and thus are less likely to develop negative intraspecific PSF compared to native species. The enemy release hypothesis has been supported by several studies showing that PSF changes from negative in the native range to neutral or positive in the secondary range for some invasive species (e.g., Callaway et al., 2004; Gundale et al., 2014; Reinhart and Callaway, 2004; Reinhart et al., 2003), or by studies showing that PSF of alien plants becomes more negative with increasing residence time due to accumulation and adaptation of local pathogens (Diez et al., 2010; Dostal et al., 2013).

It has also been shown that invasive species tend to experience more positive (Klironomos, 2002; Perkins and Nowak, 2013) or weaker negative intraspecific feedbacks than native species (Agrawal et al., 2005; Engelkes et al., 2008; MacDougall et al., 2011; van Grunsven et al., 2007). Most of the studies, however, compared intraspecific PSF of invasive species that are common in the introduced range with native species that are rare or far less common in the same area. Since PSF is known to be linked to species commonness and rarity (Kempel et al., 2018; Klironomos, 2002; MacDougall et al., 2011; van der Putten et al., 2013), it is not clear whether the observed differences in PSF between invasive and native species are more related to the species origin, or to their different abundance in the field. It has been suggested that alien species with low abundance do not benefit from PSF compared to equally abundant native species (Suding et al., 2013). However, we are not aware of any study comparing PSF of invasive and native species that are both dominant in a given area.

The results of PSF experiments are affected by conditions of plant cultivation, both biotic or abiotic, that either affect plants, soil biota or both (De Long et al., 2019; Smith-Ramesh and Reynolds, 2017; van der Putten et al., 2013; Whitaker et al., 2017). There have been calls for incorporating multiple conditions of plant cultivation into PSF experiments, as failure to consider them is likely why so many PSF experiments yield results that are either unpredictable or inconsistent between the glasshouse and the field (Heinze and Joshi, 2018; Heinze et al., 2016; Kulmatiski et al., 2008). While the effects of the biotic drivers such as competition or herbivory on the results of PSF gained quite a lot of attention in the past years (e.g., Casper and Castelli, 2007; Crawford and Knight, 2017; Heinze and Joshi, 2018; Hol et al., 2013; Muller et al., 2016; Schittko et al., 2016; Shannon et al., 2012), only a very few studies have so far focused on the role of abiotic conditions of plant cultivation on the results of PSF experiments (e.g., Florianova and Munzbergova, 2018; Fry et al., 2018; Kaisermann et al., 2017; Png et al., 2018).

Conditions of plant cultivation, such as moisture, temperature, shading or nutrient levels, are supposed to have effect on the results of PSF for several reasons. First, they represent an additional form of stress for the plants that can make them more vulnerable to negative soil effects such as to soil pathogens (Suzuki et al., 2014). Second, soil organisms respond to abiotic conditions such as temperature or moisture and their direct effects on the plants, as well as on nutrient cycling and decomposition rates, thus change accordingly (Heinze et al., 2015; Valliere and Allen, 2016; van der Putten et al., 2016). Also, the conditions of plant cultivation alter plant biomass allocation and therefore change the intensity of the interactions between plants and soil organisms. It has been suggested that PSF depends on the size and structure of root system (Bergmann et al., 2016; Cortois et al., 2016). No study has, however, specifically explored the importance of allocation to belowground biomass for the intensity of PSF.

So far, most studies addressed PSF solely during the vegetative stages of plant life (Kardol et al., 2013) with a few exceptions targeting germination, survival or establishment of seedlings (Liu et al., 2015; Mangan et al., 2010b; Packer and Clay, 2000), or production of reproductive biomass as a proxy of plant fitness (Burns et al., 2017). It has been hypothesized (Kardol et al., 2013), and shown by a few studies (Dudenhoffer et al., 2018; Florianova and Munzbergova, 2018), that PSF can change in intensity and even in direction throughout plant's life. PSF in early stages of plant life tends to be more positive than during maturity (Dudenhoffer et al., 2018) since juvenile plants have less developed root system and can thus benefit more from associations with mycorrhizal fungi (Aldrich-Wolfe, 2007; van der Heijden, 2004). Since different stages of plant life depend on different components of soil biota and different groups of soil biota respond differently to changes in abiotic conditions, we hypothesize that the effect of conditions of plant cultivation on the results of PSF will differ between life cycle stages. Evidence for this has, however, been only provided for one genus by Florianova and Munzbergova (2018) and more studies are thus needed to confirm the pattern.

In this study, we compare intraspecific PSF of three congeneric pairs of invasive and native species that are both dominant in respective ecosystems in the Czech Republic and assess the role of conditions of plant cultivation for these feedbacks in different stages of plant life. We also explore how allocation to belowground structures affects the strength of PSF. Specifically, we want to test the following hypotheses: i) Invasive species do not differ from native species with the same level of dominance in terms of PSF, ii) The results of PSF experiments are affected by cultivating conditions, iii) Cultivating conditions differ in their effect on PSF of invasive vs. native species and different performance measures, such as seedling establishment, aboveground and belowground biomass and root-shoot ratio, iv) Higher investment to belowground biomass leads to stronger PSF effects.

Materials and Methods

Study species and seed collection

For this study, we selected three pairs of congeneric species growing in the Czech Republic (Table 1). We decided to use congeners because comparisons of closely related, facultatively co-occurring, species minimize biases associated with phylogenetic distance and habitat affinities (Burns, 2004; Pyšek and Richardson, 2007). In each genus, one species is native and the other alien invasive in the country according to Pysek et al. (2012). Species within a genus were chosen to have similar physiognomy, ecological requirements in terms of Ellenberg indicator values for light, moisture, soil reaction and nutrients (Ellenberg et al., 1992), and similar level of dominance in the field in terms of maximum cover in communities according to the Pladias database (Pladias).

Seeds of all species were collected in the field in the Czech Republic in 2016. For each species, we collected the seeds from at least three different populations at least 20 km apart to account for possible intraspecific variability. The seeds collected in individual populations were then mixed because keeping them separate would highly increase the number of replicates and would make the experiment hardly feasible. In each population, we collected mature seeds from at least 10 individuals. Mother plants were not distinguished. All collected seeds were surface sterilized with 10% H_2O_2 to reduce the chance of soil contamination via seed surface fungi. The seeds of *Bidens* species were cold-wet stratified for two months prior to sowing.

Table 1: List of the studied species. Max cover refers to maximum cover the species reaches in natural communities, and grid cells to its frequency in quadrants of the basic grid mapping cells within the Czech Republic (approximately 6.0 × 5.55 km, in total ca. 2700 quadrants; both taken from Pladias).

Family	Species	Life form	Status	Max cover (%)	Grid cells
Asteraceae	*Bidens frondosus*	annual	invasive	88	1364
	Bidens tripartita		native	99	1456
Brassicaceae	*Sisymbrium loeselii*	monocarpic	invasive	88	416
	Sisymbrium officinale		native	88	1321
Onagraceae	*Epilobium ciliatum*	perennial	invasive	38	1897
	Epilobium obscurum		native	38	935

Experimental design

Following a commonly used methodology (Bever et al., 1997; Kulmatiski et al., 2008), the plants were grown in a two-phase experiment. In the first (conditioning) phase, conditioned soil was prepared. In the second (feedback) phase, intraspecific plant-soil feedback was studied. The experiment was carried out in the experimental garden of the Institute of Botany, Czech Academy of Sciences (49°59′38.972″N, 14°33′57.637″E), 320 m above sea level, temperate climate zone, where the mean annual temperature is 8.6°C and the mean annual precipitation is 610 mm.

Conditioning phase

The aim of the conditioning phase was to prepare the soil, conditioned by the species, for the upcoming feedback phase. To set up the conditioning phase, we used local soil from the experimental garden. We decided not to use soil from the field where the species grow since the genera do not co-occur and collecting soil from a variety of sites would have introduced additional variation due to soil type, fertility, pH etc. The garden soil was a good compromise to fulfill ecological requirements of all the selected species. The garden is situated in the region where the studied species occur and the local soil thus contains the soil biota commonly encountered by the species. The soil was nutrient rich ensuring that the PSF effects will not be driven by nutrient depletion of the soil. Prior to the conditioning phase, we took six samples of the soil and performed basic soil analyses (for details see Table S1).

For each species, we used 80 pots (10 × 10 × 10 cm) in the conditioning phase. Half of the pots were sown with 10 seeds of the species, the other half of the pots remained unsown and served as controls. Each pot with conditioned soil was randomly assigned its control pot in the beginning of the experiment. The pairs of pots were kept in close proximity to each other throughout the experiment to ensure they were exposed to the same conditions.

After the seeds germinated and the seedlings established, we weeded the seedlings to keep a maximum of three seedlings per pot to avoid density dependence effects between the plants but to ensure that the soil is thoroughly conditioned. Both pots with and without plants were kept under the same conditions and regularly watered. The soil was conditioned for 12 weeks, similar to a range of previous studies (e.g., Chiuffo et al., 2015; Florianova and Munzbergova, 2018; Meijer et al., 2011; van de Voorde et al., 2011; van Grunsven et al., 2007; van Grunsven et al., 2010). After the 12 weeks, all plants were harvested, divided into aboveground and belowground parts, dried to a constant weight and weighed. The biomass of the earlier removed seedlings was added to the biomass of the harvested plants.

Feedback phase

After the harvest of the conditioning phase, ten seeds of a species were sown into each pot previously conditioned by the same species as well as to the control pots. For each species we thus had 40 pots with conditioned and 40 pots with control soil with sown seeds. We did not mix the soil from all the pots conditioned by the same species within the same treatment between the conditioning and feedback phase as recommended by Brinkman et al. (2010) to avoid pseudoreplication.

In order to test for the effect of conditions of plant cultivation on the results of PSF, the plants in the feedback phase were grown under four treatments: two moisture and two shading levels in a full factorial design, i.e. moist light, moist shaded, dry light, and dry shaded. These treatments were used because drought and shading represent a common form of stress for plants. They are likely to affect plant biomass allocations and via differences in moisture and possibly temperature to have effects on soil biota. Plants in the moist treatments were watered twice a day in the morning and in the evening with tap water. Plants in the dry treatments were watered only when plant wilting was noticed except for natural rainfall. All plants of one genus were watered simultaneously once half of the plants showed signs of wilting. Shading was achieved by using a green garden shading net transmitting 65 % of incident radiation, without any significant change in the light spectrum (measured in a previous experiment by H. Skalova, unpubl.).

Due to logistic reasons, all plants exposed to the same treatment were grown together in one experimental bed. The experimental design therefore consisted of a total of four experimental beds. There were always 10 randomly selected pairs of pots with conditioned and with control soil for each species in one experimental bed, i.e. in each treatment, randomly arranged within the bed. Using this design, the treatments were technically not replicated. This should be no problem since the emphasis in this study is on the interaction between environments and plant-soil feedback. It is, however, important to keep in mind that the treatment main effects might be somewhat overestimated by random variation among the experimental beds.

To describe the differences between the treatments, one TMS3 climatic station (TOMST Co., www.tomst.com, described in Hemrova et al. (2016), was used to record temperature and moisture within each experimental bed. Soil moisture was affected both by watering regime and shading. Compared to the light moist treatment, the average soil moisture increased by 9% in the moist shaded treatment and decreased by 19% and 29% in the dry shaded and the dry light treatments, respectively. Temperature was slightly higher under the light treatments compared to the shaded treatments (Table S2).

We counted the established seedlings in all pots on weekly basis and removed the seedlings to leave only three seedlings per pot to avoid density dependence. The three seedlings were selected randomly, but in a way that ensured each seedling had enough space in the pot. When the seedlings were at least three weeks old and healthy looking, we removed all seedlings but the largest one from the pot. Seedlings emerging afterwards were counted and removed. Twelve weeks after seed germination, the plants were harvested, divided into aboveground and belowground biomass, dried to a constant weight and weighed. All individuals of the same genus were harvested from all pots simultaneously. All individuals of *Bidens* species managed to complete their life cycle within the 12 weeks. For *Bidens* species, we thus separately collected the seeds and weighed them.

Data analyses

Using the data on individual plant performance in the second phase, we calculated PSF index as ln(x/s) where x is performance of each individual plant when grown in conditioned soil and s is performance of a plant grown in the paired control pot, as suggested by Brinkman et al. (2010). An index value of less than zero indicates a negative feedback, meaning the plants perform worse in the conditioned soil than in the control soil, while a value greater than zero indicates a positive feedback, meaning the plants perform better in the conditioned soil than in the control soil. The PFS index was calculated for seedling establishment (number of established seedlings divided by the number of seeds sown), aboveground and belowground biomass, root-shoot ratio (quotient of belowground and aboveground biomass), and for *Bidens* species also for seed mass.

We analyzed the data on individual plant performance from the second phase using ANOVA with PSF index as dependent variable and genus, status (invasive, native), shading, moisture level and all their interactions as explanatory variables. Tukey HSD post-hoc tests were used for pairwise comparison of different categories. We repeated the analyses using linear mixed effect models in the R-package 'lmerTest' (Kuznetsova et al., 2017) with genus as random effect instead of a fixed effect and obtained very similar results. Because the effects of genus provide biologically relevant information, we decided to present only the ANOVA results with genus as a fixed effect in the results. We performed these analyses for PSF index for (i) seedling establishment, (ii) aboveground biomass, (iii) belowground biomass, (iv) root-shoot ratio and (v) seed mass for *Bidens* species. Data on plant performance from the conditioning phase were not considered in any analyses due to very little variation in the data. Additionally, we performed one sample t-tests for each combination of species, performance measure and treatment to see whether the PSF index for a given category significantly differed from zero. For assessing the role of biomass allocation for PSF, we used linear regression with PSF for aboveground biomass as dependent variable and average root biomass (mean value from control and conditioned soil of each pair of pots, square-root transformed to achieve normality and homogeneity of the data), along with genus, status, shading, moisture and their two-way interactions as explanatory variables. All analyses were performed using R 2.13.2 (R Development Core Team, 2014).

Results

Differences between invasive and native dominants

There was no overall difference in PSF for seedling establishment, aboveground biomass and root-shoot ratio between invasive and native species (Table 2, Fig. 1, 2, 4). PSF index for belowground biomass was significantly more negative for native species compared to invasive species (Table 2, Fig. 3). PSF index for seed mass was marginally significantly affected by species status, with the native *Bidens* having slightly more positive PSF than the invasive *Bidens.*

The t-tests performed for each species and treatment separately showed that under the moist light treatment, i.e. the treatment usually used in PSF experiments, all native species had neutral PSF for seedling establishment and only the invasive *Epilobium* had significantly positive PSF (Fig. 1). Two out of three native species showed significantly negative PSF for aboveground biomass (*Bidens* and *Sisymbrium*) and for belowground biomass (*Epilobium* and *Sisymbrium*) under the moist light treatment, while all the three invasive species showed neutral PSF for both aboveground and belowground biomass (Fig. 2, 3).

Effect of conditions of plant cultivation on the results of PSF

We found no overall effect of the conditions of plant cultivation on the result of PSF for seedling establishment (Table 2, Fig. 1). PSF index for aboveground biomass was significantly affected by moisture, shading and their interaction. PSF was generally more negative under the dry treatments compared to the moist treatments and under the light treatments compared to the shade treatments. The feedbacks of plants grown under the dry light treatment were the most negative (Table 2, Fig. 2). PSF index for belowground biomass was affected by the interaction of moisture and shading, with the index being more negative under the dry light treatment compared to the dry shaded and moist light treatments (Table 2, Fig. 3). PSF for root-shoot ratio was significantly affected by the interaction of shading, genus and status, and of shading, moisture, genus and status, showing that each species responds differently to the conditions of plant cultivation in terms of root-shoot ratio (Table 2, Fig. 4). PSF for seed mass was not affected by the conditions of plant cultivation.

Interactions of conditions of cultivation with species invasiveness and life-stage

The effect of conditions of plant cultivation on PSF significantly depended on invasive status of the species in case of PSF for seedling establishment and root-shoot ratio. The effect of status was marginally significant for PSF for belowground biomass as well (Table 2). For seedling establishment, PSF of invasive species was more positively affected by moisture than that of native species (Fig. 1). For belowground biomass and root-shoot ratio, PSF of invasive species was less negatively or more positively affected by shade compared to native species (Fig. 3, 4).

PSF of individual stages of plant life were affected differently by the conditions of plant cultivation. PSF for seedling establishment was affected only by moisture, while PSF for both aboveground and belowground biomass was affected by the interaction of shading and moisture. The main effects of shading and moisture were significant only for aboveground biomass. PSF for root-shoot ratio was significantly affected by shading and its interaction with moisture, but only in interaction with genus and status. PSF for seed mass was not affected by the conditions of plant cultivation at all (Table 2).

Table 2: Results of ANOVA testing for the effect of genus, species status (invasive vs. native), moisture, shading and their interactions on PSF index for seedling establishment, aboveground and belowground biomass, root-shoot ratio, and seed mass. The model for seed mass used only data on *Bidens* species, the effect of genus was thus not applicable (NA). Significant results ($p \leq 0.05$) are in bold, marginally significant results ($p \leq 0.1$) are in italics. Residual df = 215 for seedling establishment, aboveground and belowground biomass, and root-shoot ratio, residual df = 66 for seed mass.

		seedling establishment		aboveground biomass		belowground biomass		root-shoot ratio		seed mass	
	df	F	p	F	p	F	p	F	p	F	p
genus	2	**10.434**	**<0.001**	1.973	0.141	**5.237**	**0.006**	**9.248**	**<0.001**	NA	
status	1	0.066	0.797	0.435	0.510	**6.968**	**0.009**	2.094	0.149	*2.813*	*0.098*
moisture	1	*3.530*	*0.061*	**4.549**	**0.034**	2.485	0.116	0.003	0.960	1.261	0.266
shading	1	0.009	0.924	**6.426**	**0.012**	*2.736*	*0.099*	0.540	0.463	1.695	0.198
genus × status	2	2.259	0.107	1.122	0.327	0.062	0.940	0.385	0.680	NA	
genus × moisture	2	0.331	0.719	0.041	0.960	0.340	0.712	0.806	0.448	NA	
status × moisture	1	6.686	0.010	0.018	0.892	0.751	0.387	1.288	0.258	0.049	0.826
genus × shading	2	1.452	0.236	1.264	0.284	0.745	0.476	0.319	0.727	NA	
status × shading	1	1.491	0.223	0.001	0.973	*2.814*	*0.095*	1.725	0.190	0.051	0.822
moisture × shading	1	0.005	0.942	**3.985**	**0.047**	6.555	0.011	0.090	0.764	2.655	0.108
genus × status × moisture	2	**5.058**	**0.007**	0.361	0.697	1.230	0.294	1.016	0.364	NA	
genus × status × shading	2	0.588	0.556	1.326	0.267	1.325	0.268	**3.090**	**0.047**	NA	
genus × moisture × shading	2	*2.523*	*0.080*	1.306	0.267	0.428	0.653	0.579	0.561	NA	
status × moisture × shading	1	1.382	0.241	1.928	0.166	0.373	0.542	0.312	0.577	0.355	0.554
genus × status × moisture × shading	2	*2.342*	*0.098*	0.741	0.478	*2.499*	*0.084*	**3.305**	**0.038**	NA	

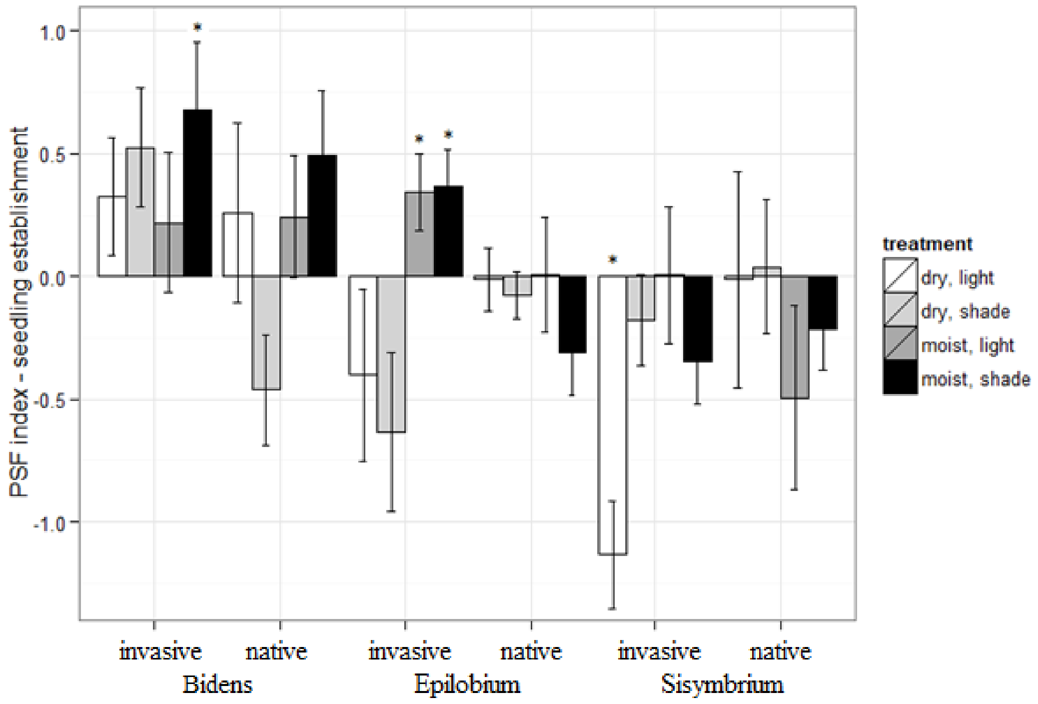

Fig. 1: Seedling establishment PSF index (mean ± SE) for individual species under all treatments. For full names of the species, see Table 1. Asterisks at bars indicate that PSF index in the given category significantly ($p \leq 0.05$) differs from zero after individual t-tests.

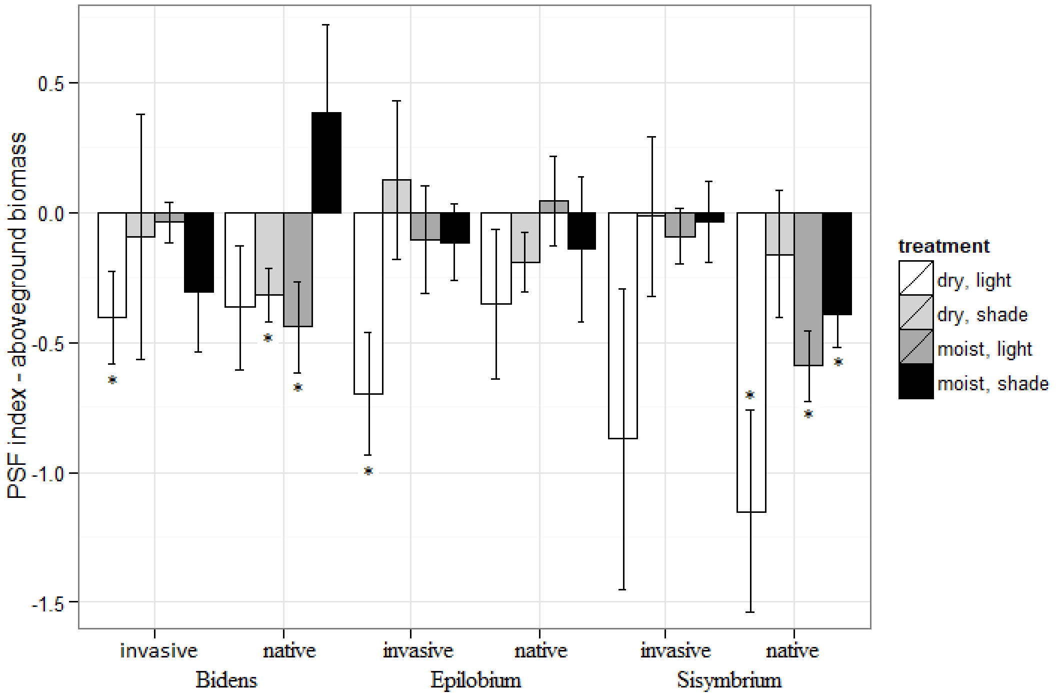

Fig. 2: PSF index for aboveground biomass (mean ± SE) for individual species under all treatments. For full names of the species, see Table 1. Asterisks at bars indicate that PSF index in the given category significantly ($p \leq 0.05$) differs from zero after individual t-tests.

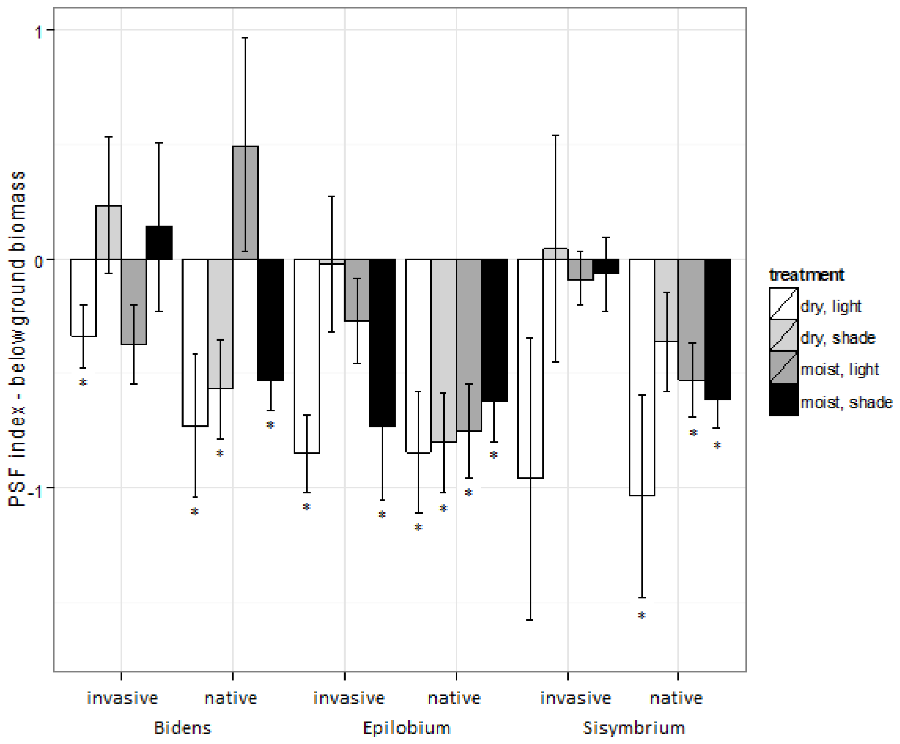

Fig. 3: PSF index for belowground biomass (mean ± SE) for individual species under all treatments. For full names of the species, see Table 1. Asterisks at bars indicate that PSF index in the given category significantly ($p \leq 0.05$) differs from zero after individual t-tests.

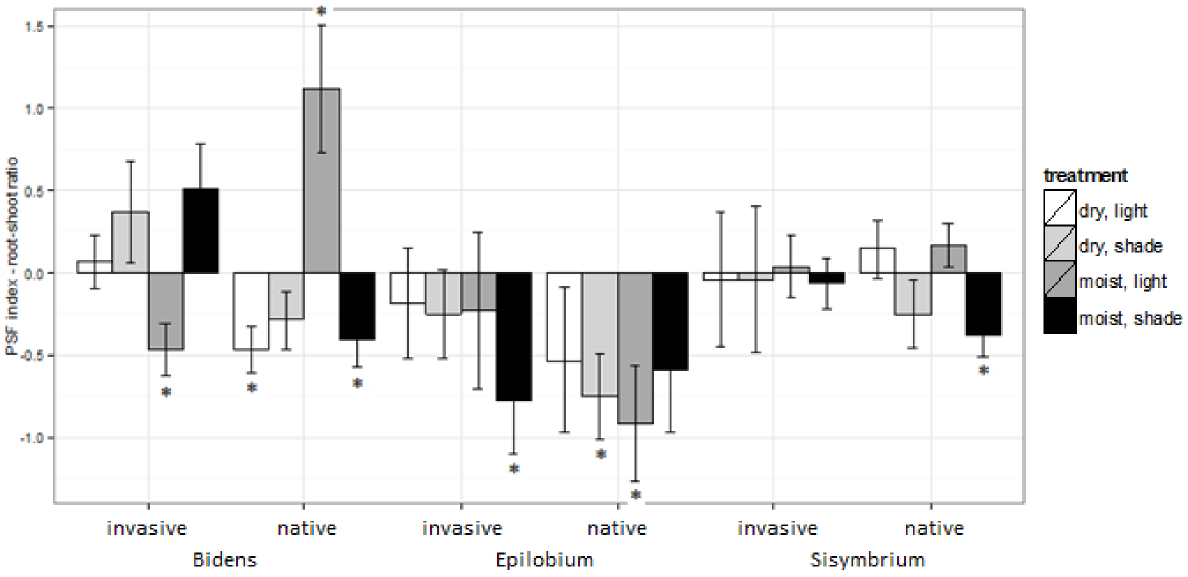

Fig. 4: Root-shoot ratio PSF index (mean ± SE) for individual species under all treatments. For full names of the species, see Table 1. Asterisks at bars indicate that PSF index in the given category significantly ($p \leq 0.05$) differs from zero after individual t-tests.

Effect of biomass allocation on PSF

PSF index for aboveground biomass was significantly negatively correlated with average root biomass ($F_{1,234} = 10.567$; $p = 0.001$; Fig. 5). This relationship remained significant when genus, status and treatments were used as covariates ($F_{1,215} = 10.802$; $p = 0.001$). No interaction of root mass with the other variables was significant ($p > 0.1$ in all cases).

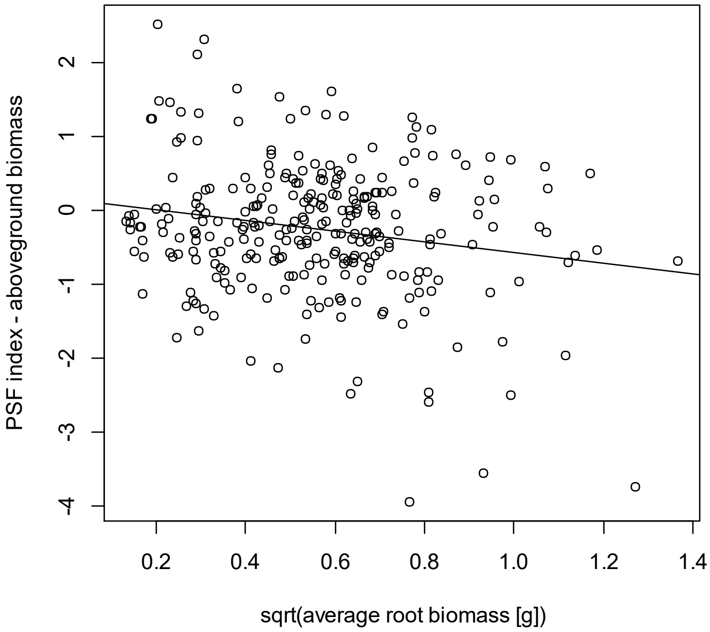

Fig. 5: Relationship between PSF index for aboveground biomass and square-root transformed root biomass (average of biomass in control and in conditioned soil).

Discussion

In this study, we compared intraspecific PSF of invasive and native plant dominants cultivated under different conditions, for different stages of plant life. We found overall difference in PSF between invasive and native dominants only for belowground biomass, where native species had more negative PSF than invasive species. We showed that conditions under which the plants are cultivated have an important effect on the results of PSF and that these effects differ among individual stages of plant life. Moreover, the effect of conditions of plant cultivation differed also between invasive and native species.

Differences between invasive and native dominants

When all treatments were considered, we only found differences between PSF of invasive and native dominants for belowground biomass. This is seemingly in contradiction to other studies that concluded that invasive species have more positive PSF for aboveground or total biomass than native species (Agrawal et al., 2005; Klironomos, 2002; Kulmatiski et al., 2008; MacDougall et al., 2011; Meisner et al., 2014; Perkins and Nowak, 2013; van Grunsven et al., 2007). However, in most of the previous studies, the invasive species were more abundant than the native species they were compared to. Our results might thus indicate that the differences found in the previous studies were possibly related to differences in plant abundance, not plant origin per se. This is in line with other studies that showed a relationship between PSF and abundance of the species, regardless of their origin (Klironomos, 2002; Mangan et al., 2010b, but see Reinhart, 2012 and Maron et al., 2016). Our result is also supported by a study of Chiuffo et al. (2015) who found no differences between PSF of coexisting native and alien, mostly non-invasive species, but reported positive relationship between PSF and cover of the species in the field.

We only focused on net outcomes of plant-soil interactions such as seedling establishment and biomass in this study and thus do not know whether the mechanisms behind these feedbacks differ between invasive and native species. The overall plant performance results from a combination of antagonistic (e.g., pathogens) and mutualistic (e.g., mycorrhiza) interactions, as well as physical and chemical properties of the soil (Reinhart and Callaway, 2006; van der Putten et al., 2013). While the invasive species might benefit from enemy release, the native species might benefit from better adaptations to local mutualists, resulting in comparable net PSF. Also, the invasive species might on one hand not be affected by some of the soil-borne pathogens in their new range and might have left their co-evolved enemies of their native range behind. On the other hand, they might be naïve and thus more vulnerable towards some of their soil-borne enemies in the new range resulting in strong detrimental effects (Parker and Gilbert, 2007; Parker et al., 2006; Verhoeven et al., 2009). Disentangling the relative contributions of different antagonistic and mutualistic soil organisms under controlled conditions would thus provide important insights into the underlying mechanisms of the feedbacks.

Smaller differences between invasive and native species in our study compared to the previous studies might also be caused by the fact that the species were chosen to have a native congener in the Czech Republic. Pathogen transmissions between phylogenetically close species are more likely than between distant species (Bufford et al., 2016; Gilbert and Parker, 2016; Parker et al., 2015; Vacher et al., 2010) and alien species having a native congener in the introduced range experience more negative intraspecific PSF compared to species not having such a close relative (Aldorfova et al. submitted).

It is important to stress that no differences between PSF of invasive and native species in our study were found only when all treatments were considered. When we focused only on the moist light treatment, i.e. the treatment used in most experimental studies, we found that native species had more negative PSF than invasive species for both aboveground and belowground biomass in two out of the three studied genera. Invasive species are usually fast-growing species with high competitive ability and lower stress tolerance (Alpert et al., 2000; Daehler, 2003). Thus, they might benefit more from growing under optimal conditions compared to the native species, but also suffer more from growing under stressed conditions. This indicates that using only the favorable conditions when studying PSF may introduce bias to native-invasive plant comparisons and artificially enhance the observed differences. Thus, it points to the importance of using multiple cultivating conditions or conditions closely resembling natural conditions in PSF experiments, as suggested by previous studies (Florianova and Munzbergova, 2018; Heinze et al., 2016).

Effect of conditions of plant cultivation on the results of PSF

Conditions of plant cultivation had an important effect on the results of PSF, which corresponds with conclusions of a range of previous studies (e.g., De Long et al., 2019; Fry et al., 2018; Kaisermann et al., 2017; Png et al., 2018; Smith and Reynolds, 2015; Smith-Ramesh and Reynolds, 2017). In this study, we focused on the effect of moisture and shading, and similar to some studies, we only applied the conditions in the feedback phase. Using this approach allowed us to detect context dependence in plant's ability to respond to existing soil mutualists and pathogens in the soil and/or of the cultivating conditions on the soil microbial community, but it did not provide information on how the conditions influence plant effects on the soil (Smith-Ramesh and Reynolds, 2017).

The effect of shading has so far been very rarely studied, even though it has been suggested to play an important role in PSF (Smith-Ramesh and Reynolds, 2017). It has been suggested that increasing light may result in stronger, more negative PSF due to increased nutrient limitation and greater allocation to belowground biomass (Smith and Reynolds, 2015; Smith-Ramesh and Reynolds, 2017). However, PSF may also become less negative or more positive with increasing light (Smith-Ramesh and Reynolds, 2017) due to higher specificity and allocation to mutualists (Mangan et al., 2010a) that drive positive PSF. Increased light may also be less favorable for soil pathogens (Augspurger, 1984; Augspurger and Kelly, 1984; McCarthy-Neumann and Ibanez, 2013) that drive the negative PSF. Only three previous studies dealt with the effect of light availability on results of PSF, one providing evidence for PSF becoming more negative under high light (Smith and Reynolds, 2015), similarly to the present study, one providing evidence for negative PSF effects being restricted to shaded conditions only (McCarthy-Neumann and Ibanez, 2013), and one showing no net effect of shading (Florianova and Munzbergova, 2018). In our study, as well as in Florianova and Munzbergova (2018), the effect of shading on PSF interacted with the effect of moisture, and in our study was most likely explained by altered biomass allocation (see below). These results indicate that the effect of shading on the results of PSF is largely context dependent, changes with other environmental variables and depends on whether the PSF is driven by abiotic factors, pathogens or mutualists.

Moisture can affect the results of PSF via changes in soil microbial communities, altered soil nutrient availability and by altering plant biomass, and the effects of moisture on results of PSF have been shown to be species specific (Fry et al., 2018; Kaisermann et al., 2017). Our study showed that the effect of moisture also depends on plant life stage. There was no general pattern in the effect of moisture on PSF for seedling establishment. *Bidens* species had more positive PSF under the moist treatments compared to the dry treatments, while the pattern was the opposite in *Sisymbrium* species. These differences might be explained by different ecological requirements of the genera. *Bidens* species require wet, often soaked, poorly aerated soils and grow mostly on shores of rivers or lakes and wetland margins. On the contrary, *Sisymbrium* species prefer soils of lower to average moisture, avoiding wet soils as well as soils that frequently dry out, and grow mostly on termophilous ruderal sites, such as road sides or forest edges (Pladias). These findings are thus in line with our hypothesis that PSF effects get more positive under favorable conditions compared to conditions where the plant is simultaneously stressed by the environmental conditions (Suzuki et al., 2014). Results of PSF for aboveground biomass were generally more negative under the dry treatments compared to the moist ones, but the effect of interaction of moisture and shading was also significant. PSF was more negative under both moisture extremes, i.e. dry light treatment and moist shaded treatment, indicating increased susceptibility of plants to soil pathogens under moisture extremes (Kaisermann et al., 2017; Suzuki et al., 2014). The strong negative effect of cultivating conditions on results of PSF under the dry light treatment in our study is further likely facilitated by increased allocation of biomass to roots and thus intensified interactions with soil biota.

Interactions of conditions of cultivation with species invasiveness and life-stage

The effect of conditions of plant cultivation on results of PSF differed between invasive and native species, suggesting that PSF of the two groups of plants is determined by different mechanisms. Similarly, the effects of cultivating conditions differed between individual stages of plant life, indicating that individual components of PSF are of different importance for juvenile and for adult plants (Kardol et al., 2013) and further pointing to the neccessity of considering multiple stages of plant life cycle in PSF experiments (Dudenhoffer et al., 2018; Kardol et al., 2013).

Effect of biomass allocation on PSF

It has been suggested that the size and structure of root system affect the intensity of PSF via affecting the intensity of interactions between the plant and the soil biota, especially soil pathogens. Cortois et al. (2016) and Bergmann et al. (2016) showed that PSF is negatively correlated with specific root length and that the effects of soil pathogens are more pronounced in plants with high specific root length. In our study, we showed that PSF for aboveground biomass is negatively correlated with root mass and we propose that increased biomass allocation and thus more intense interactions of the plant with soil pathogens is responsible for more negative PSF effects under the dry and light treatments, as has been previously suggested by Smith and Reynolds (2015).

Methodological constrains

When interpreting the results of this study, it is important to bear in mind its limitations. First, we only studied three pairs of species and therefore cannot generalize the conclusions on differences in PSF between invasive and native dominants. However, despite the low number of species, our results indicate that the conditions of plant cultivation affect whether differences in PSF between the two groups of species are detected or not.

The results of PSF experiments are largely affected by the initial soil used (van de Voorde et al. 2012). In our study, we used local garden soil while the majority of previous studies used soil collected directly in the localities. Our soil could thus initially contain lower abundances of species specific biota that would cause its slower accumulation during the cultivation phase and consequently weaker PSF effects. However, the character of the feedback should remain unchanged. The method of treating the control soil during the cultivation phase can also affect the results of PSF experiments (Brinkman et al., 2010). While some studies use soil conditioned by another species or by a mixture of species as a control, we used unconditioned soil exposed to the same conditions (similarly to Florianova and Munzbergova, 2018; Kardol et al., 2007; Kulmatiski et al., 2011; Perkins and Nowak, 2013; Wang et al., 2013). This approach can cause the PSF to be more negative due to nutrient depletion of the conditioned soil and this effect might be species specific. Altering the level of nutrients is, however, also part of PSF, even though usually ignored in the experiments, and we reduced the likelihood of nutrient depletion by using nutrient rich soil.

Conclusions

Our study pointed to some problems rarely addressed in comparisons of PSF between invasive and native species, namely considering invasive and native species with the same level of dominance in the field, involving multiple conditions of plant cultivation and studying PSF in different stages of plant life. We showed that PSF of juvenile plants may differ not only in the intensity, but also in the direction from that of adult plants, and that conditions of plant cultivation play an important role for PSF. We also showed that invasive and native species with the same level of dominance in the field do not generally create more positive PSF than native dominant species. This indicates that differences in PSF between invasive and native species reported in previous studies could have been caused by different level of dominance in the field and not by plant origin per se. It is also possible that the differences only exist under some conditions of plant cultivation. Further investigations are needed to confirm the generality of the patterns. More detailed studies focusing on the role of soil biota and soil chemistry would help us to understand the mechanisms underlying the feedbacks that might differ for invasive and native dominants and that might cause the differences in PSF response to conditions of plant cultivation. Last, we suggested that the strength and intensity of PSF is affected by altered plant allocation to belowground biomass under different conditions of plant cultivation, as the more biomass the plant allocated to the roots, the more negative PSF it generated.

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Supporting Information

Table S1: Abiotic characteristics of the used soil prior to the conditioning phase. Values show the mean and stardard deviation of six samples. The analyses were performed by the Analytical Laboratory of Institute of Botany, Czech Academy of Sciences, Průhonice. The methods used for the analyses are described in detail in Raabova et al. (2008).

Soil characteristic	mean ± sd
$pH(H_20)$	7.77 ± 0.03
pH(KCl)	7.7 ± 0.01
total N [%]	0.07 ± 0.02
total C [%]	1.01 ± 0.2
exchangeable Ca [mg/kg]	1259.35 ± 125.63
exchangeable Mg [mg/kg]	105.03 ± 11.53
exchangeable K [mg/kg]	278.6 ± 21.61
exchangeable P [mg/kg]	39.99 ± 1.12
total P [mg/kg]	159.46 ± 46.7

Table S2: Mean temperature and moisture under individual treatments during the feedback phase. The data were recorded from 12th July to 12th October every 15 min. Soil temperature was measured 6 cm below soil surface, air temperature 10 cm above soil surface.

treatment	month	temperature (ºC)			soil moisture
		soil	surface	air	
dry, light	July	21.63	21.25	21.29	1397.30
	August	18.24	18.58	18.20	1217.32
	September	17.02	17.17	16.98	1067.13
	October	9.21	8.55	8.50	1396.60
dry, shade	July	21.81	21.25	21.19	1625.31
	August	17.74	18.00	17.89	1422.18
	September	16.48	16.67	16.58	1272.70
	October	9.59	8.54	8.33	1450.96
moist, light	July	21.40	21.50	21.37	1772.05
	August	17.81	18.37	18.21	1639.52
	September	15.55	16.20	16.49	1818.13
	October	8.40	8.22	8.33	1931.58
moist, shade	July	20.48	20.64	20.90	2004.19
	August	17.33	17.44	17.71	1897.65
	September	15.83	16.05	16.33	1863.48
	October	9.19	8.40	8.35	2007.66

Study 3

The intensity of intraspecific plant-soil feedbacks in alien *Impatiens* species depends on the environment

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Abstract

Plant-soil feedback, i.e. the relationship in which a plant affects the composition of the soil and such modified soil affects plant growth, is becoming an important concept for explaining plant invasiveness. *Impatiens parviflora* is one of the most widespread invasive plant species in Central Europe, but it is unknown whether this species exhibits any form of plant–soil feedback, previously seen in other invasive species. In this study, we examined intraspecific plant-soil feedback of *I. parviflora* and compared it to feedbacks of other three alien, but non-invasive, *Impatiens* species growing in Central Europe. Moreover, we studied the effect of environmental conditions on this feedback.

The four species were studied in a two-phase feedback experiment. In the first phase, soil was conditioned by a species or left unconditioned (control). During the second phase, plants were grown in soil conditioned by the same species and in control, not cultivated soil, under four different environmental conditions (two levels of watering × two levels of shading).

All the studied species showed positive plant-soil feedback in some types of environment indicating they can potentially become invasive. The feedbacks for total biomass and for root-shoot ratio were significantly affected by environmental conditions. Individual species and studied performance measures responded differently to individual treatments. In most cases, the feedback was changing from positive in optimal treatment to neutral and in *I. balsamina* to negative under some suboptimal treatments. No effect of the environmental conditions on the feedback for germination was observed.

These results indicate that environmental conditions play an important role in plant-soil feedbacks. We show that some feedbacks detected in highly controlled experiments using just one type of environmental conditions might not exist in the field, and similarly some existing feedbacks might remain undetected. We therefore recommend using multiple environmental conditions or conditions closely resembling conditions in natural sites to increase the realism of the results.

Key words: congener; drought; plant invasiveness; shading; small balsam; stress.

Introduction

Plant-soil feedback, the process in which a plant affects biotic and abiotic soil characteristics and the soil in turn affects growth of the plant, is becoming an important concept for explaining invasiveness of introduced species (Dostálek et al., 2016; Levine et al., 2006; Meisner et al., 2014). At the interspecific level, a range of studies demonstrated that invasive species can benefit from suppressing the growth of other species via production of alellopathic substances secreted into the environment, depletion of nutrients necessary for the growth of other plants, or changes in composition of soil communities (e.g., Del Fabbro and Prati, 2015; Mangla et al., 2008; Shannon et al., 2014; van der Putten et al., 2013; Wardle et al., 1998; Yang et al., 2014). At the intraspecific level, it has been demonstrated that invasive plants often show positive or less negative intraspecific plant-soil feedback compared to native species (Kulmatiski et al., 2008) which may allow them to become dominant in their new environment (Klironomos, 2002).

However, most alien species do not become invasive in their new range (Williamson and Fitter, 1996) and stay in casual or naturalized stage. Casual species are able to flourish and occasionally reproduce, but are not able to form self-replacing populations and rely on repeating introductions for their persistence. Naturalized species reproduce consistently and sustain populations over many life cycles without direct intervention by humans. Invasive species are a subset of naturalized species that form dense populations and spread over large areas (Richardson et al., 2000). While positive intraspecific plant-soil feedback has been documented for many invasive species, it is not clear whether alien non-invasive species also exhibit positive intraspecific plant-soil feedback and their invasion is limited by some other traits such as time since introduction or frequency of planting, or if their plant-soil feedback is negative and prevents them from becoming invasive. Most studies dealing with the importance of plant-soil feedback for plant invasiveness either compare plant-soil feedback of invasive species in their native and new range, or compare plant-soil feedback between invasive and native species. We are aware of a very few studies comparing directly plant-soil feedback between invasive and alien non-invasive species (e.g., Kempel et al., 2013; Zuppinger-Dingley et al., 2011) or comparing plant-soil feedback of alien species in areas where they are invasive with areas where they are alien but not invasive (e.g., Andonian et al., 2011).

Impatiens parviflora is one of the most widespread invasive plant species in Europe, occurring in 34 European countries in total (Lambdon et al., 2008). It is most frequent in Central Europe where it behaves as a dominant species in many types of habitats (Pyšek et al., 2012b; Sádlo et al., 2007). Although most aspects of its biology are known, it is not sure what sort of plant-soil feedback is exerted by the species. Positive feedback might be expected, as it was previously shown by other invasive species (e.g., Coykedall and Houseman, 2014; Gundale et al., 2014; Maron et al., 2014; Nijjer et al., 2008; Yang et al., 2013). In this study, we will examine intraspecific plant-soil feedback of *Impatiens parviflora* and compare it with other alien, but non-invasive, *Impatiens* species.

Common limitation of plant-soil feedback experiments is that plants are grown in highly controlled greenhouse or common garden experiments under favorable conditions. A plant growing in optimal conditions (e.g., sufficient water and light availability, no competition) might perform well even though the soil in which it is growing is unfavorable, while under stress conditions it might be more vulnerable to negative soil effects such as soil pathogens. Along these lines, previous studies showed that the outcome of plant-soil feedback experiments is affected by competition (Casper and Castelli, 2007; Hol et al., 2013; Shannon et al., 2011) and foliar herbivory (Schittko et al., 2016).

However, only a few studies have been dealing with the role of environmental conditions. Environmental conditions such as soil moisture, light intensity and temperature can affect the outcome of plant-soil feedback experiments for three reasons. First, these factors can influence soil microbial communities (reviewed in van der Putten et al., 2016) that have various impacts on plant growth. This is supported by study of Heinze et al. (2015) where biotic soil effects were affected by temperature (but see also Lau and Lennon, 2011 or van Grunsven et al., 2010 for no effect of temperature). It is also in line with Valliere and Allen (2016) who showed differences in root colonization by both mycorrhizal and non-mycorrhizal fungi in different watering and nitrogen deposition treatments. Second, the environmental factors can affect plant biomass allocation and therefore change the interactions with soil organisms. For example, under dry conditions, we expect the plant-soil feedback to be more important because plants allocate more biomass to roots and therefore interact with the soil more intensively than under moist conditions. Along these lines, McCarthy-Neumann and Kobe (2010) demonstrated that the vulnerability of *Acer rubrum* and *Fraxinus americana* seedlings to soil pathogens decreased when grown in shaded conditions compared to not shaded conditions. Third, environmental conditions might affect the way plants cope with negative effects of soil pathogens. When the plant grows under optimal conditions and has sufficient amount of resources, it might be better able to fight against the pathogens and overcome their negative effect. When it is stressed, the negative part of the feedback might be more pronounced. Since the plants often grow under various environmental conditions in the field, it is important to assess plant-soil feedback over multiple environments the plants can experience and compare the results.

The main aims of this study are to examine intraspecific plant-soil feedback in *Impatiens parviflora*, compare it to intraspecific plant-soil feedback of other alien, but non-invasive, *Impatiens* species and assess the importance of environmental conditions for this plant-soil feedback. To do so, we performed a two-phase common garden experiment (plants were grown in pots) for four *Impatiens* species. We were using species of one genus because comparisons of closely related facultatively co-occurring species minimize biases associated with phylogenetic distance and habitat affinities (Burns, 2004; Caldwell et al., 1981; Pyšek and Richardson, 2007). In the first phase of the experiment, we planted the plants under standard conditions (enough light and water) to obtain a conditioned soil. In the second phase, we planted the plants in either the conditioned or control (not conditioned) soil under four treatments (environmental conditions). The treatments included a full factorial combination of two watering and two shading regimes. We decided to use the various treatments only in the second phase of the experiment because we primarily wanted to see if the plant reactions to the same soil differ under different environmental conditions. Thus, we only used optimal conditions in the first phase so that all the soil was cultivated in the same way. However, an alternative approach using different treatments in the first phase that would focus mostly on differences in soil communities might also bring interesting findings.

Specifically, we aim at answering the following questions: i) What is the intraspecific plant-soil feedback in individual *Impatiens* species?, ii) Does the invasive *I. parviflora* have more positive intraspecific plant-soil feedback that the other three *Impatiens* species?, iii) Are the results of plant-soil feedback experiments affected by environmental conditions?, iv) Are there any differences in the effect of environmental conditions on plant-soil feedback between species?, v) Are all performance measures commonly studied in plant-soil feedback experiments (germination, total biomass, root-shoot ratio) affected by environmental conditions in the same way?

Material and Methods

Studied species and seed collection

For this study, we selected four annual alien *Impatiens* species, one of which is invasive (*I. parviflora*) and three casual (*I. balsamina*, *I. balfourii* and *I. scabrida*) in the Czech Republic, Europe (Pyšek et al., 2012a). All species are native to Asia, with *I. balsamina* coming from subtropical and tropical areas of India and the other three species from temperate areas of Central Asia (Tabak and von Wettberg, 2008). All species were introduced to Europe as ornamental plants (Pyšek et al., 2012a). *I. parviflora* and *I. balfourii* have very similar ecological demands in terms of soil moisture and climate. Both species prefer humid soils and do not tolerate really dry or water-logged soils. They are both adapted to warm climate with optimal temperatures for net photosynthesis between 24 and 32°C and do not tolerate temperatures below zero. However, their requirements for light availability are different. *I. parviflora* prefers shaded conditions and is able to tolerate shade up to 10 % of incident radiation. *I. balfourii* prefers higher light intensities, which allows the species to grow outside forests in open habitats (Schmitz and Dericks, 2010). Ecological requirements of *I. scabrida* are not well known, but based on its distribution it is expected to prefer moist soils and shaded conditions. Unlike the two previously described species, *I. scabrida* is adapted to cold climate and is able to withstand temperatures below zero. *I. balsamina* is classified as a subtropical species and does not tolerate frosts. It is known to be highly susceptible to drought (reviewed in Matthews et al., 2015).

For *I. parviflora*, which is very common in the Czech Republic, we collected mature seeds at three localities from at least 10 individuals per locality in 2014. The seeds from individual localities were mixed and considered as one universal source of seeds as growing them separately would lead to very high number of pots in the experiment. For all other species, we obtained mature seeds from at least 10 individuals grown in the experimental garden of Institute of Botany, Academy of Science of the Czech Republic within a previous experiment (Čuda et al., 2016). Mother plants were not distinguished. Collected seeds were stored in paper bags till the start of the experiment.

Conditioning phase

Following commonly used methodology (Bever et al., 1997; Kulmatiski et al., 2008), the plants were grown in a two-phase experiment. In the first (conditioning) phase, conditioned soil was prepared. In the second (feedback) phase, intraspecific plant-soil feedback was studied. The experiment was carried out in the experimental garden of Institute of Botany, Academy of Sciences of the Czech Republic (49°59′38.972″N, 14°33′57.637″E), 320 m above sea level, temperate climate zone, where the mean annual temperature is 8.6°C and the mean annual precipitation is 610 mm.

The aim of the conditioning phase was to prepare the soil, conditioned by the species, for upcoming feedback phase. To set up the first phase, we used a common garden soil mixed with sand in 1:1 ratio. We decided not to use soil from the field where the species grow since the non-invasive *Impatiens* species are very rare in the field and do not form monotypic stands. The soil would thus be largely affected by other species occurring on the site and the effect of *Impatiens* cultivation might not be detected. For each species, we used 80 pots (10 × 10 × 10 cm) in the conditioning phase. Half of the pots were sown with 10 seeds of the species, the other half of the pots served as controls. The seeds were cold-wet stratified for two months prior to sowing. After the seeds germinated and the seedlings established, we weeded the seedlings to keep a maximum of 3 seedlings per pot and thus avoid density dependence effects between the plants but ensure that the soil is thoroughly conditioned. Both pots with and without plants were kept under the same conditions and regularly watered. The soil was conditioned for 12 weeks, similar to a range of previous studies (e.g., Meijer et al., 2011; van de Voorde et al., 2011; van Grunsven et al., 2007; 2010). After the 12 weeks, all plants were harvested, divided into aboveground and belowground parts, dried to a constant weight and weighed. The biomass of the earlier removed seedlings was added to the biomass of the harvested plants.

Feedback phase

After the harvest of the conditioning phase, the soil from each pot was sieved to remove all remaining root fragments and was placed back in the same pot. The soil from control pots was sieved as well to minimize differences between the soil treatments. Ten seeds of the same species were then sown into each previously conditioned pot as well as to the control pots. For each species we thus had 40 pots with conditioned and 40 pots with control soil with sown seeds. We did not mix the soil from all the pots conditioned by the same species within the same treatment between the conditioning and feedback phase as recommended by Brinkman et al. (2010) to avoid pseudoreplication.

In order to test the effect of environmental conditions on plant-soil feedback, the plants in the feedback phase were grown under four treatments: two moisture and two shading levels in a full factorial design, i.e. moist + light, moist + shade, dry + light, and dry + shade. Plants in the moist treatments were watered twice a day in the morning and in the evening with tap water. Plants in the dry treatments were watered only when plant wilting was noticed except for natural rainfall. All plants were watered simultaneously once half of the plants showed wilting. Shading was achieved by using a green garden shading net transmitting 65 % of incident radiation, without any significant change in the light spectrum. Due to logistic reasons, all plants exposed to the same treatment were grown together in one experimental bed. The experimental design therefore consisted of a total of four experimental beds. There were always 10 pots with conditioned and 10 pots with control soil of each species in one experimental bed, i.e. in each treatment. Using this design, the treatments were technically not replicated. We believe it is not a problem since the emphasis is on the interaction between environments and plant-soil feedback. It is, however, important to keep in mind that the treatment main effects might be somewhat overestimated by random variation among the experimental beds. To describe the exact differences between the treatments, TMS3 climatic stations (TOMST Co., www.tomst.com, described in Hemrová et al., 2016) were placed in each experimental bed.

We followed germination of the seeds in the pots. To avoid density dependence, we counted and removed the seedlings to leave only three seedlings per pot. The three seedlings were selected randomly, but in a way that ensured each seedling had enough space in the pot. After at least some of the plants established (they were at least three weeks old and healthy looking) we removed all seedlings but the largest one from each pot. Seedlings emerging afterwards were counted and removed. Twelve weeks after seed germination, the plants were harvested, divided to aboveground and belowground biomass, dried to a constant weight and weighed. All plants of the same species were harvested from all pots simultaneously. The biomass of the removed seedlings was added to the biomass of the harvested plants and the total biomass was used in further analyses. However, we performed all the analyses also without the biomass of removed seedlings and we got nearly identical results (not shown).

Data analyses

Using the data on individual plant performance in the second phase, we calculated the index of plant-soil feedback (PSF index) as ln(x/s) where x is performance of each individual plant when grown in conditioned soil under a certain treatment and s is mean performance of plants grown in control soil under the same treatment (i.e. average value from the ten control pots in given treatment). According to Brinkman et al. (2010), this method results in a less conservative test than dividing each conditioned soil by an individual control, however, it is the second best option in case such independent controls do not exist. An index value of less than zero indicates a negative feedback, meaning the plant performs worse in conditioned soil than in control soil, while a value greater than zero indicates a positive feedback, meaning the plant performs better in conditioned soil than in control soil. The index was calculated for total plant biomass (summed aboveground and belowground biomasses), root-shoot ratio and germination (here used as number of emerged seedlings divided by number of seeds sown). We did not use separate indices for individual aboveground and belowground biomass as the biomasses were highly correlated to each other ($R^2 = 0.77$) and thus also to total biomass ($R^2 = 0.97$ for aboveground and $R^2 = 0.85$ for belowground biomass).

We analyzed the data on individual plant performance from the second phase using ANOVA with PSF index as dependent variable and invasive status (casual, invasive), species identity, shading and moisture levels and all their interactions as explanatory variables. We performed these analyses for PSF index for (i) total biomass, (ii) root-shoot ratio and (iii) germination. Data on plant performance from the first phase were not considered in any analyses due to very little variation in the data. Additionally, we performed one sample t-tests for each combination of performance measure, species and treatment to see whether the PSF index for a given category significantly differed from zero. For each performance measure, we performed 16 individual tests. Thus, we applied the Bonferroni correction and reduced the conventional p-level from 0.05 to 0.003 (Dunn, 1961). Since the Bonferroni correction is considered too conservative by many authors (e.g., García, 2004; Moran, 2003) and many studies decided not to use any correction for this reason (e.g., Bowman et al., 2008; Münzbergová, 2007; Scheepens and Stocklin, 2013), we report and illustrate results both with and without this correction. All analyses were performed using R 2.13.2 (R Development Core Team, 2014).

Results

Both germination (Fig. A1) and total biomass (Fig. A2) of plants grown in control soil were the highest under moist light treatment for all species. This treatment was therefore considered as the optimal treatment in further text. Data from climatic sensors showed that average temperatures in the light experimental beds were approximately 0.5°C higher than average temperatures in the shaded experimental beds, independent of watering regime. Soil moisture was affected both by watering regime and shading. Compared to the optimal treatment, moist shaded experimental bed experienced 7% increase in soil moisture and dry shaded and dry light experimental beds 21% and 32% decrease in soil moisture, respectively.

The main effect of invasive status was significant only for the germination PSF index, with the invasive *I. parviflora* having lower index than the non-invasive *Impatiens* species. The main effect of species identity was significant only for the root-shoot ratio PSF index, with *I. parviflora* and *I. balfourii* having higher index than *I. balsamina* and *I. scabrida*. The main effects of moisture and shading were not significant for any of the studied performance measures. The interaction of moisture and shading significantly affected the total biomass PSF index and marginally significantly ($p < 0.1$) the germination and root-shoot ratio PSF indices. In addition, the interaction of species, moisture and shading was significant for the total biomass PSF index and marginally significant for the root-shoot ratio PSF index. The interaction of invasive status, moisture and shading was significant for the root-shoot ratio PSF index (Table 1).

Environmental conditions had only a marginally significant effect on germination PSF index but some differences between treatments and individual species were visible (Fig. 1). *I. parviflora* showed neutral feedback for germination under all treatments indicating that soil conditioning had no effect on germination regardless of the environmental conditions. *I. balfourii* germinated better in conditioned soil than in control soil when grown under moist light treatment, but neutral feedback was detected in the other treatments. Contrary to this, *I. balsamina* and *I. scabrida* showed positive intraspecific feedbacks for germination under all treatments except for one, namely moist shaded and moist light, respectively. After the Bonferroni correction, the only germination PSF index significantly different from zero was that of *I. balsamina* under dry light treatment.

Total biomass PSF index was significantly affected both by interaction of moisture and shading and the triple interaction of species, moisture and shading. *I. balfourii*, *I. balsamina* and *I.*

parviflora had positive total biomass PSF index when grown under moist light treatment. *I. balfourii* and *I. balsamina* showed positive biomass feedback effect also when grown under dry shaded treatment, but neutral feedback effects were detected under the remaining treatments except for *I. balsamina* which showed negative feedback under moist shaded treatment. *I. scabrida*, on the contrary, showed positive feedback under dry light and moist shaded treatments and neutral feedback under dry shaded and moist light treatments (Fig. 2). The only biomass PSF index that remained significant after the Bonferroni correction was the negative PSF index for *I. balsamina* under moist shaded treatment.

Table 1: Results of ANOVA testing for the effect of invasive status, species, moisture, shading and their interactions on PSF index for germination, total biomass and root-shoot ratio. Significant results ($p \leq 0.05$) are in bold, marginally significant results ($p \leq 0.1$) are in italics. Residual df = 116.

		germination		total biomass		root-shoot ratio	
	df	F	p	F	p	F	p
invasive status	1	**5.692**	**0.019**	0.208	0.649	2.316	0.131
species	2	0.514	0.600	0.619	0.540	**3.549**	**0.032**
moisture	1	0.001	0.973	1.717	0.193	0.004	0.952
shading	1	0.603	0.439	*3.642*	*0.059*	0.079	0.780
moisture×shading	1	*3.373*	*0.069*	**6.233**	**0.014**	*3.049*	*0.083*
invasive status×moisture	1	0.005	0.945	0.056	0.813	**6.690**	**0.011**
invasive status×shading	1	0.380	0.539	0.039	0.844	0.049	0.826
species×moisture	2	2.211	0.114	0.676	0.511	0.040	0.961
species×shading	2	0.392	0.676	1.887	0.156	0.090	0.914
invasive status×moisture×shading	1	0.287	0.593	0.089	0.767	**5.633**	**0.019**
species×moisture×shading	2	0.927	0.399	**4.609**	**0.012**	*2.502*	*0.086*

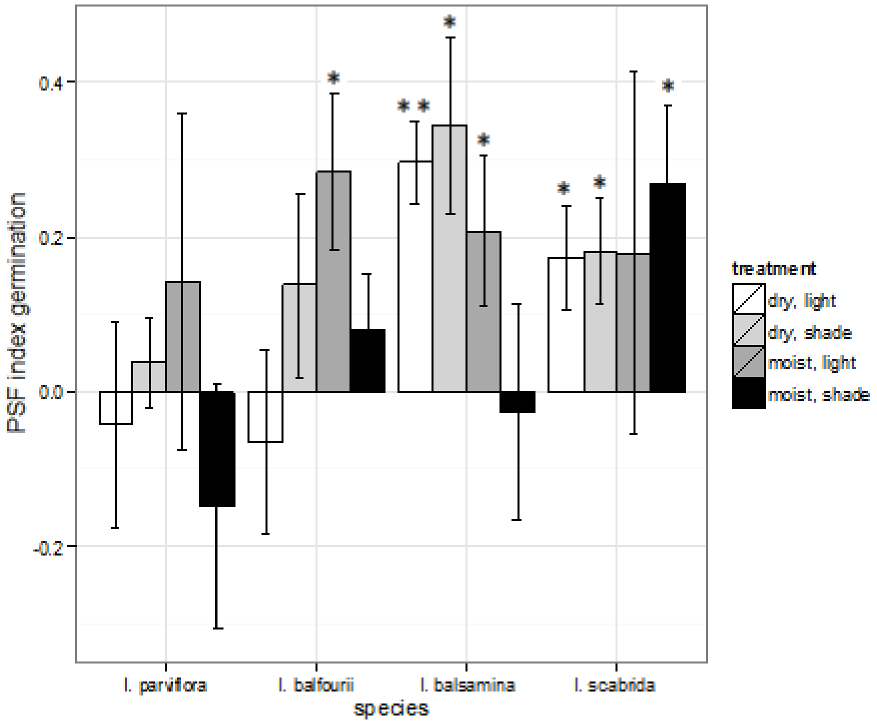

Fig. 1: Germination PSF index (mean ± SE) for individual species under all treatments. * indicate that PSF index in the given category significantly ($p \leq 0.05$) differs from zero after individual t-tests, ** indicate the index differs from zero after the Bonferroni correction was applied.

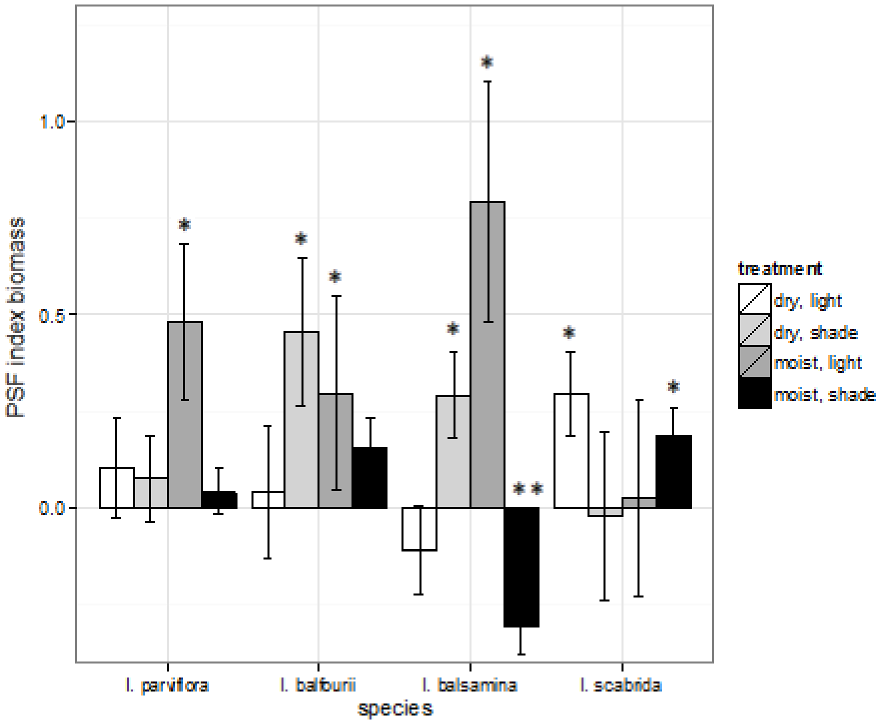

Fig. 2: Total biomass PSF index (mean ± SE) for individual species under all treatments. * indicate that PSF index in the given category significantly ($p \leq 0.05$) differs from zero after individual t-tests, ** indicate the index differs from zero after the Bonferroni correction was applied.

The root-shoot ratio PSF index was significantly affected by interaction of species, moisture and shading. The index did not significantly differ from zero in *I. balsamina* and *I. scabrida* under any treatment. *I. balfourii* had positive root-shoot ratio PSF index under dry shaded and moist light treatments and neutral under the remaining two treatments. *I. parviflora* showed positive feedback under all treatments except for moist light treatment where it was neutral (Fig. 3). No root-shoot ratio PSF index significantly differed from zero after the Bonferroni correction was applied.

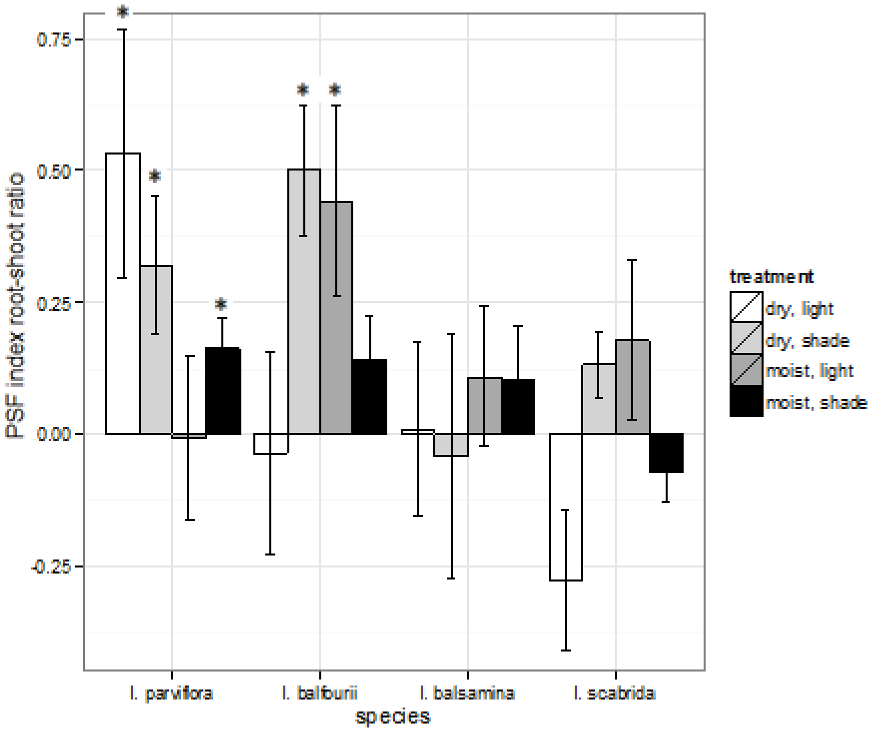

Fig. 3: Root-shoot ratio PSF index (mean ± SE) for individual species under all treatments. * indicate that PSF index in the given category significantly ($p \leq 0.05$) differs from zero after individual t-tests.

Discussion

All *Impatiens* species in our study showed positive plant-soil feedback (PSF) in some types of environments, regardless their invasive status. For *Impatiens parviflora*, this result was expectable as it is an invasive species being dominant in several habitats in the Czech Republic (Pyšek et al., 2012b; Sádlo et al., 2007). It is in line with Pattison et al. (2016) who detected positive intraspecific PSF in closely related invasive *I. glandulifera*. It is also supported by range of other studies showing positive PSF in invasive species (e.g., Coykedall and Houseman, 2014; Gundale et al., 2014; Maron et al., 2014; Nijjer et al., 2008; Yang et al., 2013).

Positive PSF in the three non-invasive species means they do not differ from the invasive species in terms of PSF and might have the potential to become invasive as well. However, the three non-invasive species may differ in many other traits from the invasive species (see Richardson and Pyšek, 2006 and van Kleunen et al., 2010). Moreover, naturalization success and invasive spread is strongly affected by time since introduction (Pyšek and Jarošík, 2005) and propagule pressure (Colautti et al., 2006; Čuda et al., 2016). All of the studied non-invasive *Impatiens* species were introduced in the Czech Republic later than *I. parviflora* (Pyšek et al., 2012a) and are not frequently cultivated (Čuda et al., 2016). It is therefore possible that these species possess an intrinsic invasiveness that, due to differences in invasion history, may not have reached its full potential yet. It might be especially the case of *I. balfourii.* It has been shown to possess the same ecophysiological traits as the highly invasive *I. glandulifera* and *I. parviflora* (Ugoletti et al., 2011), seems to be well adapted to local environmental conditions (Schmitz and Dericks, 2010), and presents traits linked to high attractiveness for pollinators and high fecundity (Jacquemart et al., 2015). The spread of this species should thus be carefully monitored.

However, when interpreting the ecological implications of the detected positive feedbacks in *Impatiens* species, we must be very cautious and realize the limitation of the study. In this study, we used common garden substrate. It means that we were testing how the *Impatiens* species respond to a novel soil with microbes that can be considered naïve in their response to the plants. This corresponds to the situation when the species colonizes a new habitat, but does not tell much about the longer-term effects of soil biota on these plants. It has been shown that the effect of soil on non-native plants changes over time and the feedbacks typically become more negative due to soil microbes becoming adapted to the new plant species (Diez et al., 2010; Dostál et al., 2013). The feedbacks in established populations of the *Impatiens* species might therefore differ from the results reported here.

The positive PSF detected in our studied species might originate from accumulation of beneficial arbuscular mycorrhizal fungi and thus improved acquisition of phosphorus to the plants (Richardson et al., 2009). Since *Impatiens* species are known to be mycorrhizal (Štajerová et al., 2009) and the performance of *I. balsamina* (Bañuelos et al., 2012) and *I. parviflora* (Chmura and Gucwa-Przepióra, 2012) has been shown to be improved by presence of arbuscular mycorrhizal fungi, this explanation is quite likely. However, Pattison et al. (2016) showed reduced colonization by arbuscular mycorrhizal fungi in soils conditioned by *I.*

glandulifera compared to control soil. Similarly, Tanner and Gange (2013) showed reduced mycorrhizal colonization in *Plantago lanceolata* and in *Lotus corniculatus* when grown in soils previously dominated by *I. glandulifera*, and Rucklii et al. (2014) showed the same for *Acer pseudoplatanus*.

Another mechanism possibly causing the positive PSF in our studied species may be increased phosphorous or nitrogen levels via root exudation or differences in the soil microbial community, as seems to be the case of *I. glandulifera* positive intraspecific PSF (Pattison et al., 2016). Based on our data, we are, however, not able to distinguish between the possible determinants of the feedback. More detailed investigations studying the soil biota composition and nutrient levels are therefore needed.

The PSF was significantly affected by environmental conditions, i.e. interaction of moisture and shading treatments. In most cases, the feedback was positive under dry shaded and moist light treatments, and neutral or even negative under moist shaded and dry light treatments. This is in line with the hypothesis that plants might overcome negative effects of soil more easily under favorable conditions and thus exhibit more positive feedback than in suboptimal environments. Both moist light and dry shaded treatments might be considered favorable for *Impatiens* as *Impatiens* are known to tolerate high levels of shading (Čuda et al., 2014; Peace and Grubb, 1982; Schmitz and Dericks, 2010) and as water shortage in dry shaded treatment was not striking due to decreased rate of evapotranspiration. Contrary to this, under dry light and moist shaded treatments the plants were probably suffering from too low and too high levels of soil moisture, respectively. Suboptimal conditions in moist shade and dry light treatments are indicated by the fact that the total biomass (Fig. A2) of all *Impatiens* species and germination of some of the species (Fig. A1) in control soil were the lowest in these treatments.

In this study, environmental conditions were not only affecting PSF for total biomass representing overall plant performance, but also PSF for root-shoot ratio. Plants can adapt to variation in resource availability by partitioning their biomass allocation to structures that acquire a limiting resource (Poorter and Nagel, 2000). Under the drought treatment, plants are therefore likely to increase relative allocation to root biomass, while under the shading treatment they are likely to increase relative allocation to shoot biomass. This is, however, likely to happen both in conditioned and control soil, therefore it should not cause changes in the PSF index. There are two possible reasons for changes in PSF for root-shoot ratio with environmental conditions in our study. Firstly, root-shoot ratio responds also to nutrient levels which differ between conditioned and control soil (higher levels of nutrients in control soil as no nutrients were used up during the conditioning phase), and the effect of nutrient availability can interact with the effects of environmental conditions. This is supported by Valliere and Allen (2016), who showed that intraspecific PSF for root-shoot ratio in *Artemisia californica* decreased under drought treatment compared to well-watered treatment under high nitrogen deposition but did not change under low nitrogen deposition. Secondly, differential allocation responses have also been detected when confronting plants with arbuscular mycorrhizal fungi (Pánková et al., 2011; Streitwolf-Engel et al., 1997), soil pathogens (D`Hertefeld and van der Putten, 1998) or soil microbes of different geographical origin (te Beest et al., 2009). As soil microbial communities likely differ between conditioned and control soil and as they are likely to respond differently to environmental conditions (van der Putten et al., 2016), microbial communities may explain the difference in PSF for root-shoot ratio between individual treatments.

The effect of environmental conditions on plant-soil feedback for germination was only marginally significant and it differed from effects shown by total biomass in both magnitude and direction. This is in line with other studies who found contrasting effects of soil on different stages of plant life (e.g., Brandt et al., 2013; Deck et al., 2013; Münzbergová and Šurinová, 2015). Furthermore, studies from different fields of plant ecology demonstrated differential effects of various ecological factors on different stages of plant life (e.g., Dostálek and Münzbergová, 2013; Knappová et al., 2013; Münzbergová et al., 2013).

Under different treatments, plant responses to conditioned soil differed not only in the intensity, but also in the direction of the feedback effects. In most species, we detected both positive and neutral feedback depending on the treatment and in case of *I. balsamina* even positive, neutral and negative feedback. Even though the moist light treatment can be considered an optimum for our model species, it does not represent the conditions under which these species typically occur in nature (Čuda et al., 2014; Matthews et al., 2015). It is therefore possible that positive PSF detected in some species under moist light treatment may be artifacts of unusually favorable conditions and do not really exist in natural systems. This is supported by Heinze et al. (2016), who compared both intra- and interspecific PSF of selected species in the greenhouse and in the field and showed that all PSFs were more positive in the favorable conditions in the greenhouse than in the field. It is also in line with Schittko et al. (2016), who found positive intraspecific PSF in five out of eight studied species when grown in the greenhouse but neutral

PSF when the plants were moved to the field where they were exposed to increased herbivore pressure. These results show the risk of commonly performed greenhouse or common garden experiments that only use favorable conditions. Using just one type of environmental conditions means that some PSF existing in natural habitats may not be detected and that significant PSF that do not exist in natural habitats could be falsely detected. The question of the ecological relevance of experiments conducted under well-watered conditions even when using drought adapted species was raised also by Valliere and Allen (2016), who observed dramatic differences in the direction and magnitude of intraspecific feedback in *Artemisia californica* when grown under well watered and drought treatments, especially when the plants were exposed to high nitrogen deposition.

The effect of environmental conditions on PSF differed between species, even though these species belong to the same genus and have quite similar ecological requirements. The response to environmental conditions was affected by the autecology of the planted species, with the two most ecologically similar species *I. balfourii* and *I. parviflora* showing the most similar response to the treatments. It is therefore not possible to generalize our results and suggest one particular type of environmental conditions to be used in all further experiments. We suggest that multiple environmental conditions or environmental conditions closely resembling conditions in natural sites should thus be used in future experiments to increase the reliability of the results.

In this study, we only focused on modifying the environmental conditions in the feedback phase of the experiment. It allowed us to detect changes in plant growth caused by changes in the way plants cope with either positive or negative effect of soil including effects of composition, diversity and abundances of soil microbial communities and soil chemistry during the feedback phase. However, studying the effect of environmental conditions in the conditioning phase could also provide interesting results. While some PSF studies use soil from the field as conditioned soil, many studies perform the conditioning phase of the experiment in a greenhouse or a common garden (Kulmatiski et al., 2008). In that case, soil conditioning under favorable experimental conditions can significantly differ from actual soil conditioning in natural systems as both soil microbes and nutrient availability are known to be affected by soil moisture and temperature (reviewed in van der Putten et al., 2016). Along those lines, Meisner et al. (2013) demonstrated the effect of a drought treatment prior to the feedback phase on the composition of soil biota and PSF of selected native and alien species.

Our study shows the importance of environmental conditions for results of PSF experiments. The effect of biotic conditions, for example plant competition, on the outcome of PSF experiments has already been shown by previous studies, with very similar results – both direction and magnitude of PSF can change in the context of competition and these changes are species specific. For example Shannon et al. (2012) showed negative intraspecific PSF in *Microstegium vimineum* when grown in a competitive mixture but neutral PSF when grown separately, and Hol et al. (2013) showed that competition by weeds increased the sensitivity of cereals to negative intraspecific PSF effects. Casper and Castelli (2007) showed that intraspecific feedback of *Sorghastrum nutans* was clearly expressed in the absence of competition. It was, however, eliminated by introducing interspecific competition into the experimental design. As competition itself is known to be affected by environmental conditions (Čuda et al., 2015; Skálová et al., 2013) including water availability (Franzese and Ghermandi, 2014), shading (Molina-Montenegro et al., 2012) or nutrient supply (Powell and Knight, 2009), competition and environmental conditions are likely to have interactive effect on PSF. It is therefore advisable to conduct more complex PSF experiments including both competition and environmental forms of stress to better understand the nature of the process and to allow us to appraise the level of bias in the results obtained by simplified greenhouse or common garden experiments.

Conclusions

The key finding of our study is that the outcome of PSF experiments is affected by environmental conditions, and that magnitude and direction of the change differ between species and studied performance measures. Our results indicate that some feedback detected in highly controlled conditions of greenhouse or common garden might not exist under natural conditions. Similarly, some feedback existing in the field can remain undetected. Therefore, we recommend using multiple environmental conditions or conditions closely resembling those found in natural sites in future PSF experiments to increase the realism of the results.

Our study is also the first study showing positive intraspecific PSF in *Impatiens parviflora* which might contribute to its massive spread throughout Europe. In addition, we showed positive PSF also in other alien, but non-invasive, *Impatiens* species, which might mean they have the potential of becoming invasive as well. Therefore, other plant traits related to plant invasiveness should be studied in these species to predict their potential future invasion and their spread should be monitored. The PSF experiments using these species should also be repeated in natural conditions as our data describe PSF at the stage of initial stand colonization, but do not allow to describe the processes in established *Impatiens* stands.

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Appendix A

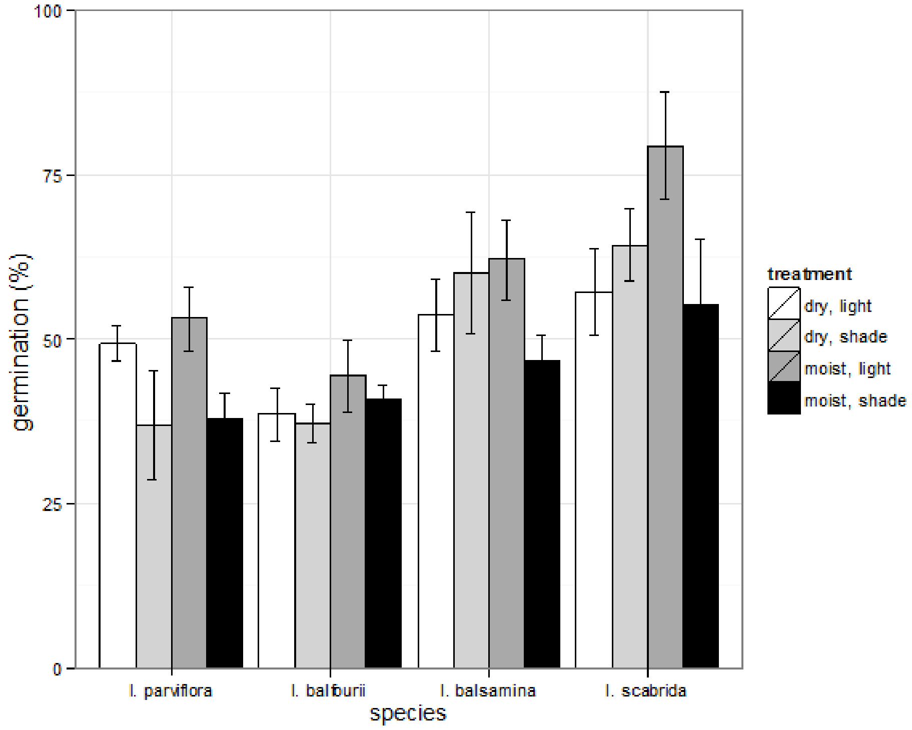

Fig. A1: Germination (mean ± SE) of seeds grown in control soil for individual species under all treatments.

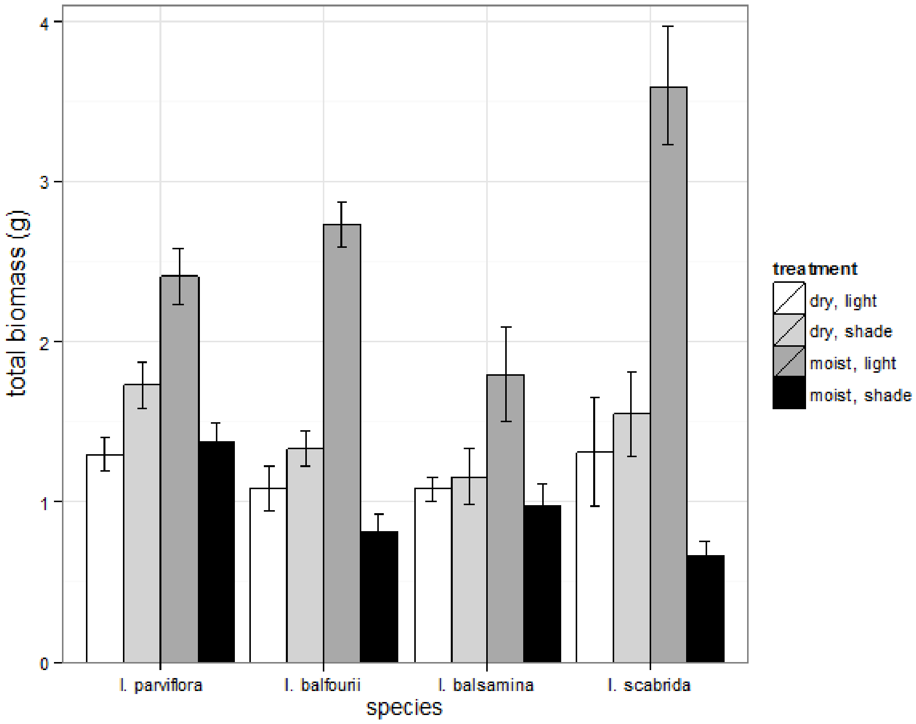

Fig. A2: Total biomass (mean ± SE) of plants grown in control soil for individual species under all treatments.

Study 4

Drivers of natural spread of invasive *Impatiens parviflora* differ between life-cycle stages

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Abstract

Impatiens parviflora is one of the most widely spread invasive species in central Europe, yet the factors affecting its spread are still subject to discussion. The aim of this study was to determine which factors affect the spread of *I. parviflora.* This was achieved by observing the natural spread of the species from 2012 to 2016 in 15 permanent transects in six different habitats within the Czech Republic. The transects were divided into 321 plots, whilst data on environmental conditions, as well as of the spread of *I. parviflora* and its performance, were collected in each plot.

The results showed that individual stages of the *I. parviflora* life-cycle were affected by individual environmental conditions to different extents. The most important factor preventing seedling emergence and establishment was a high cover of herb layer. It did not, however, affect survival of older plants. Thus, *I. parviflora* can grow in sites with dense cover of herb layer in case the cover formed after *I. parviflora* seedlings established. Juvenile mortality was the highest in sites with low nutrient levels and low soil moisture. Canopy openness had a negative effect on *I. parviflora* performance. *I. parviflora* performed better in neutral soils, in comparison to acidic soils.

Oak-hornbeam forests were the most suitable habitat for *I. parviflora*, followed by acidophilous oak and mixed coniferous forests. However, *I. parviflora* was able to penetrate into even species-rich habitats, such as thermophilous oak forests, as well as steppe grasslands on rocks. This makes it a potential threat to biodiversity. Only heathlands found on former pastures proved to be unsuitable for *I. parviflora,* as these remained uninvaded until the end of the study.

Key Words: Balsaminaceae; central Europe; habitat requirements; natural spread observation; small balsam.

Introduction

Understanding the causes and consequences of plant invasions is one of the key topics in plant ecology. Invasive plants affect natural communities. They displace native species and change vegetation structure, whilst additionally causing a reduction in diversity within the affected areas (Hejda et al. 2009, Powell et al. 2013). Furthermore, they undermine the functionality of whole ecosystems (Richardson and Pyšek 2012) and cause significant economic losses (Zavaleta et al. 2000). Most previous studies focus on the impact that invasive species have on invaded habitats, or on traits underlying plant invasiveness. However, it is also important to study the environmental factors that affect the spread of individual invasive species, so that we can predict which habitats or areas are likely to be invaded by a given species and, possibly, inform as to how we can prevent the invasion (Higgins et al. 1996).

Most of the studies dealing with factors affecting the spread of invasive species and their ecological requirements are based on comparisons of invaded and non-invaded habitats (space-for-time substitution, Pickett 1989; Chmura et al. 2007, Godefroid and Koedam 2010). However, these studies are limited by several problems. First, they do not consider the possibility that the species did not have enough time to enter the habitat. Second, many species can modify the environmental characteristics of the invaded habitat. Using a simple comparison of invaded and non-invaded habitats cannot, thusly, distinguish between the causes and consequences of the invasion. This approach would also not reveal a situation when the species invades the habitat with certain environmental conditions. For example, the environmental conditions could later change, due to factors other than the invasion of the species itself (e.g., succession, disturbance) and the species just has not been displaced yet (Rejmánek et al. 2013). Lastly, this approach does not allow for disentangling between individual phases of invasion. Absence of a species in a habitat can be caused by many different factors, for example: lack of seed availability in the area; seeds not being able to germinate; seedlings not being able to establish; plants not being able to complete their life cycle; plants not producing enough seeds; all of which result in different implications for the management of any given invasive species.

One of the methods that allows for a determination of factors which affect the spread of an invasive species, without the above-mentioned complications, is to observe its spread into a new habitat from the very beginning. We can either use a sowing experiment (for example Becerra and Bustamante 2011, Cheplick 2010, Christen and Matlack 2009), i.e. sow a given number of seeds of the invasive species into a selected habitat and record its performance, or we can follow the natural spread of the species in newly invaded areas. A sowing experiment has two significant advantages over natural spread observation. First, it allows for the controlling of a number of propagules in the experimental plots. Second, it allows for the selection of as many habitats and replications within a habitat as we need. However, we need to carry the responsibility of introducing invasive plants in non-invaded natural habitats. Therefore, observing the natural spread of a species is more suitable, even though the interpretation of the obtained data is more complicated. To observe the spread of an invasive species, we have to find an area where the species is not yet widely dispersed, but where it is in the infancy of spreading. To minimize the uncertainty about propagule numbers entering the habitat, we should know the distance of the selected habitat from the source population (e.g., Crosti et al. 2016, Langdon et al. 2010, Miller and Matlack 2010, Soukupová 1992).

In the present paper, we aim to assess factors which affect the spread of *Impatiens parviflora*. *I. parviflora* is one of the most widely spread invasive species in central Europe (Lambdon et al. 2008) that colonizes a wide spectrum of habitats (Pyšek et al. 2012, Sádlo et al. 2007), in addition to having a weak, yet negative, overall effect on native vegetation (Diekmann et al. 2016, Florianová and Münzbergová 2017). However, factors affecting the spread of *I. parvifora* are still not clear. According to most authors, *I. parviflora* prefers disturbed habitats which possess a low cover of native species (Eliáš 1992, 1999, Faliński 1998, Chmura and Sierka 2006, Kujawa and Pawlaczyk 1991, Obidzinski and Symonides 2000). Further, it is said that *I. parviflora* prefers shaded habitats, along with sufficient soil moisture and high levels of nutrients (Coombe 1956, Čuda et al. 2014, Ellenberg et al. 1992). Futhermore, its spread is limited by drought (Csontos et al. 2012) and frosts (Skálová et al. 2011). However, some authors point out that *I. parviflora* also invades open, dry, as well as nutrient poor habitats with high cover of native species (Godefroid and Koedam 2010, Stepień 2009).

Since most previous studies dealing with factors that affect the distribution of *I. parviflora* and its ecological requirements were based on comparisons of invaded and non-invaded habitats, it is not possible to disentangle factors affecting its spread and its ecological requirements from the consequences of its invasion. Therefore, we aim to determine which factors affect the spread of *I. parviflora* by observing the natural spread of the species. The spread of *I. parviflora* was monitored during 2011-2016 in permanent plots found in Central Bohemia, Czech Republic, within an area where – according to previous observations – the species had just started to spread. The spread was observed in transects orthogonal to source population (i.e. path), with the source population at the transect beginning. Transects were placed in different habitats. These include: oak-hornbeam forests, thermophilous and acidophilous oak forests, mixed coniferous forests, heathlands on former pastures, and dry grasslands on rocks. In all transects, we observed the spread and performance of *I. parviflora* and recorded various environmental factors. Such an approach allowed us to assess the importance of individual environmental factors on individual life cycle stages, observe the invasion process, and help with answering the following questions: (i) What is the temporal dynamics of *I. parviflora* spread in different habitats? (ii) What are the factors affecting the different demographic rates (seedling emergence and establishment, survival to maturity, fitness) of *I. parviflora*?

Methods

Study species

Impatiens parviflora is an annual representative of the Balsaminaceae family, native to eastern and central Asia. The species was first introduced to Europe in 1831 as an ornamental plant (Coombe 1956). Since this time, it spread throughout the whole of Europe, except for the very south and north of the continent (Trepl 1984). *I. parviflora* has been reported from 34 European countries in total (Lambdon et al. 2008), with central Europe being one of the most widely invaded regions. In the Czech Republic, it was first introduced in 1844 and became naturalized in the 1870s (Slavík 1997). The species is common over the whole area of the Czech Republic, except for very dry and mountainous areas (Pyšek et al. 2012).

I. parviflora is known as a species with variable ecological requirements (Chmura et al. 2007, Godefroid and Koedam 2010), one that is able to colonize a wide spectrum of habitats with various environmental characteristics (Eliáš 1992, Piskorz and Klimko 2007, Sádlo et al. 2007, Tokarska-Guzik et al. 2008). In the Czech Republic, it has been recorded in 45 types of habitats. In a range of these habitats – such as in nitrophilous herb vegetation of mesic sites or in herb layer in alluvial, ravine or oak-hornbeam forests – it is considered a dominant species. In *Robinia pseudoacacia* plantations, it is considered a constant dominant, as it determines the general appearance of the habitat and occurs in more than 40 % of the localities of the habitat (Pyšek et al. 2012).

I. parviflora germinates in the beginning of April and has very fast growth rate in the early phases of its life. The period of flowering and fruiting is very long, extending from June to November (Ellenberg 1996). Flowers are pollinated by small Syrphidae and Apidae, but are capable of autonomous selfing as well (Vervoort et al. 2011). The fruits are autochor capsules with one to five seeds (Csiszár and Dénes 2008, Slavík 1997). Under optimal conditions, one plant can produce up to 2000 seeds per season (Csiszár and Dénes 2008, Trepl 1984). The seeds are spread by autochory and can be dispersed to a distance up to 3 to 4 meters (Trepl 1984). Humans may also disperse the seeds over long distances (Vervoort and Jacquemart 2012). The species has a limited seed bank (Perglová et al. 2009) and, as an annual, is thus crucially dependent on the successful establishment of juveniles every year.

Study sites and experimental design

The study was carried out in the nature reserve Zvolská Homole, Central Bohemia, Czech Republic (49°56’24’’N, 14°24’09’’E). This area was chosen because (i) it comprises many types of habitats on a small area (47 ha), which allows us to study the role of environmental factors without confounding variables such as climate, and (ii) because according to previous observations, *I. parviflora* just started spreading into this area circa. 2010.

Factors affecting the spread of *I. parviflora* were studied in sites that were not massively colonized by *I. parviflora* and where only solitary individuals, or small groups of *I. parviflora,* grew along the paths. Permanent transects were established in these sites in August 2011. The transects were one meter wide, orthogonal to the path as the origin of *I. parviflora* spread. Transects always went from the source *I. parviflora* population to at least ten meters past the last *I. parviflora* occurrence. The length of the transects, therefore, varied from 14 to 44 m (on average 21.4 m). The length of the transects remained unchanged throughout the experiment. Each transect was divided into 1×1 m plots and, in each plot, numbers of *I. parviflora* individuals were counted. Fifteen transects – a total length of 321 m – were established amongst six types of habitats. Three transects were located in steppe grassland on rocks, four in heathlands on former pastures, three in thermophilous oak-forest, one in acidophilous oak forest, two in oak-hornbeam forest, and two in mixed coniferous forest. In 2011, *I. parviflora* was present in 100 plots, while it was missing in 221 plots (for details see Appendix 1).

Data on I. parviflora spread

The number of *I. parviflora* individuals in the plots were counted every spring (last week of April) and summer (third week of July), from 2012 – 2016. The number of *I. parviflora* individuals in spring allowed us to assess the ability of seeds to germinate as well as seedlings to establish. Since the germination requirements of *I. parviflora* are already known (see Perglová et al. 2009, Skálová et al. 2011), in addition to it also being difficult to study germination in the field, we focused on seedling emergence and establishment. As such, we were counting *I. parviflora* plants in spring as late as in last week of April, even though the seeds generally start to germinate in the first half of April (Ellenberg 1996). The number of detected plants was therefore significantly lower than number of all germinated seeds. The data represented only the plants that were able to survive, approximately, the first two weeks of their development, and those that were usually already quite large (up to 15 cm). The number of *I. parviflora* individuals in the summer represented the number of individuals that reached maturity and were able to produce seeds.

Every summer, the total *I. parviflora* cover was estimated. The estimates were rounded to units of percent at plots with less than 10 % of *I. parviflora* cover and to tens of percent at plots with higher *I. parviflora* cover. Height and number of fruits of the three biggest individuals were recorded in each plot in 2013-2016. The number of fruits was used as an estimate of *I. parviflora* fitness. The number of fruits does not precisely reflect a plant's reproductive effort, as individual fruits may have a different number of seeds, whereas the seeds may also be of different quality. However: assessing the number of seeds, or seed mass, was impossible due to observing the natural spread of the species, and thus the need to affect its reproduction as little as possible.

Data on environmental conditions

Canopy openness and slope were measured in each plot in July 2013. Canopy openness served as a proxy of light availability and was estimated based on hemispherical photographs (Guevara-Escobar et al. 2005). We used a digital Canon 350D camera with objective Sigma 8 mm F3.5 EX DG Circular Fisheye with default camera settings. The camera was situated in a vertical position, in the plot center, one meter above the surface, with the bottom of the photograph situated to the north. To minimize error due to direct sunlight, we took photos in the early morning or late evening, when the sun was low above the horizon. Photographs were converted in the program SideLook (Nobis 2005) to bitmaps using blue color channel, whereby we achieved the maximum contrast between tree canopies and the sky. The bitmap image was then analyzed in the program Gap Light Analyzer 2.0 (Frazer et al. 1999). Canopy openness was measured only once within the study. We assume it did not change between years as there were no visible changes in the structure of the studied habitats in given years (e.g., no fallen or cut down trees), and the plots did not undergo any intensive successional change.

Soil moisture was measured three times every year between April and August using a Moisture meter HH2 device with probe Theta Probe ML29 (Delta-T Devices, UK). Moisture was measured in all four corners of each plot and average values were calculated. To prevent bias in the data, moisture was always measured on a day following a week without any untypical weather, such as intense rainfall or extreme drought. We used an average value from all four corners and all years for soil moisture in the analyses.

In July 2013, soil samples were taken from the upper soil horizon (0–5 cm) at the edge of each plot to avoid damage to the plants in the plot. Soil pH(H_2O) was measured with the use of a combined glass electrode WUW SenTix 41 with automatic temperature calibration.

Each plot was further divided into nine 33 × 33 cm subplots and phytocenological relevés were taken in each subplot in July 2012. Subplots were used to make the estimates of the cover more precise; however, we only worked with average values for the whole plots in the analyses. All vascular plant species and their cover were recorded. The estimates were rounded to units of percent for plants with less than 10 % cover and to tens of percent for plants with higher cover. Mosses and lichens were not determined, for only their total cover was recorded. Based on this data, number of species, the summarized cover of the herb layer and the summarized cover of graminoids were determined. The summarized cover of the herb layer represents the sum of covers of all species in the herb layer, except for *I. parviflora.* Summarized cover of graminoids represents sum of covers of all species from Poaceae, Cyperaceae and Juncaceae family. Summarized covers were used as a proxy of above-ground biomass, as opposed to the commonly used herb layer cover, as the significance of the herb layer cover-biomass relationship gradually declines towards denser vegetation (see also Axmanová et al. 2012). Cover of the herb layer represented a proxy for the level of disturbance in our plots (the higher the cover, the lower the disturbance). We believe that the cover of herb layer reflects the level of aboveground disturbance well, such as grazing by deer, which is the most common form of disturbance in the studied area. No belowground disturbances caused for example by digging animals or uprooted trees were recorded in any transect during the experiment, so the effect of such events on *I. parviflora* spread could not be studied. Since the number of species, summarized cover of herb layer and summarized cover of graminoids were strongly correlated (see Appendix 2 for details), only summarized cover of herb layer was used in further analyses.

Using species data obtained from the plots (with *I. parviflora* excluded), we calculated the mean Ellenberg indicator values (EIV) for light, moisture, soil reaction, nutrients, temperature and continentality (Ellenberg et al. 1992). The calculations were weighted by species abundances, as this provides more accurate results than simple presence-absence data on species composition (Schaffers and Sýkora 2000). EIV, for temperature and continentality, were excluded from further analyses because of the minimum variability between plots. EIV for light, moisture and soil reaction, were strongly correlated with direct measurements of canopy openness, soil moisture and soil reaction, respectively (see Appendix 2 for details). They were therefore excluded from further analyses. Thus, only EIV for nutrients was used in the analyses.

Statistical analyses

We first calculated a pair-wise correlation matrix with all measured variables describing the environmental conditions (Appendix 2) and excluded all highly correlated variables from further analyses. In all further analyses, we only used five environmental characteristics as explanatory variables: canopy openness, EIV for nutrients, soil moisture, soil reaction and summarized cover of herb layer (hereafter as herb layer cover).

Differences in environmental characteristics between transects (Appendix 3) were studied using linear direct gradient analysis (Redundancy Analysis, RDA) and Monte-Carlo permutation tests (ter Braak and Šmilauer 1998) with 499 permutations. Dependent variables used in this analysis were the five environmental characteristics (see above), and were standardized prior to the analysis. The independent variable was transect identity. The analysis was performed in Canoco 5 (ter Braak and Šmilauer 2012).

Next, we studied the factors affecting the presence of *I. parviflora* and the number of individuals in spring and in summer, juvenile mortality (i.e. proportion of individuals present in a plot in spring that did not survive till summer), and the number of fruits (average of the three individuals measured in a plot). Factors affecting plant height and cover were not studied, since plant height and cover were highly correlated to each other and to number of fruits.

Due to large differences between the transects, as well as low variation in some variables within the transects, all of the data was analyzed in two steps: first at the transect level, second at the plot level with transects used as covariates. At both transect and plot level, we used generalized mixed effect models in "lme4" R package (Bates et al. 2015). Transect identity was used as random effect in transect level analyses, plot identity in plot level analyses. Binomial error distribution was used for models studying presence of *I. parviflora* in spring and in summer, and for juvenile mortality. Poisson error distribution was used for number of individuals in spring and in summer and for number of fruits. Year was used as a discrete covariate in all models.

Explanatory variables used in all analyses were the five environmental characteristics described above. In plot level analyses, we included interactions of these variables and year. In transect level analyses, these interactions were not considered due to low number of degrees of freedom. Optimal model, i.e. model with the highest explanatory power was selected with function "dredge" in "MuMIn" R package (Bartoń 2009). The function is using second-order Akaike information criterion (AICc) to rank the models and to obtain model weights. Since the approach requires computing results for each possible model, we needed to reduce the number of explanatory variables in the plot level analyses. To do so, we first tested for the effect of each explanatory variable and each variable × year interaction separately and excluded all variables that were not significant. This approach was not needed for transect level analyses since we did not consider the interactions of environmental characteristics and year and thus the total number of possible models was not so high. To estimate the p-values for variables selected for the optimal model, we used F-tests comparing two models with and without the tested term.

In plot level analyses, the model for presence of *I. parviflora* used presence in previous years as a covariate. For the number of individuals, only subset of plots where *I. parviflora* was present was used for the analysis. The number of individuals in the previous year was used as a covariate. For juvenile mortality, as well as for the number of fruits, we only used plots where *I. parviflora* was present in spring and summer, respectively.

In all plot level analyses, distance from the path (i.e. distance from the source population) was used as a covariate to account for the different number of propagules entering individual plots and/or the intensity of disturbance at the plot. Since the seed supply/disturbance does not have to decrease linearly with increasing distance from the source population, we first tested which function of distance from the path (simple value, square root and logarithm) explains the most variability in number of individuals in plots in subsequent years. Simple distance from the path explained more variability in the data than any other function and was thus used in the analyses.

For visualization, we selected the two best predictors from each dependent variable based on deviance explained by the given predictor (the higher the explained deviance, the better the predictor) – from this we drew contour plots based on GLM prediction models for them. We considered both plot level and transect level analyses when selecting the two most important predictors for each demographic rate; usually the best predictor at the transect level, and the best predictor at the plot level, were selected for visualization of the given demographic rate. The contour plots are based on the plot level data, but without transects being filtered out, meaning that differences at the transect level are visible as well.

All the univariate analyses were performed in R 3.1.0 (R Development Core Team 2014).

Results

Spread in different habitats

Impatiens parviflora successfully spread within most of the studied transects between 2011 and 2016. The number of plots invaded by *I. parviflora* in summer increased from 100 in 2011 to 196 in 2016. The increase was mostly continual, with two declines in 2012 and 2015, probably due to exceptionally dry springs. These two years also showed the largest difference between numbers of plots invaded in spring and in summer, showing that there was greater mortality in these years than in any other year (Appendix 1).

The greatest increase in number of invaded plots was observed in transects located in oak-hornbeam forests and mixed coniferous forests. In these transects, *I. parviflora* spread into all studied plots within two years after the beginning of the study. In the four transects located in heathlands on former pastures, no increase or a decline in number of invaded plots was observed. All remaining transects (i.e. steppe grasslands on rocks, thermophilous and acidophilous oak forests) experienced an increase in number of invaded plots that did not reach 100 % plot occupancy within the transect (Appendix 1). In oak-hornbeam forests, mixed coniferous forests and acidophilous oak forest, *I. parviflora* formed dense populations of up to

40-50 individuals per plot. In steppe grasslands on rocks and in thermophilous oak forests, it only reached densities of 5-10 individuals per plot (Appendix 4).

Effect of environmental conditions

Presence of *I. parviflora* in spring at the transect level was negatively affected by canopy openness and herb layer cover, and positively affected by EIV for nutrients, soil reaction and soil moisture (Table 1). At the plot level, presence of *I. parviflora* in spring was positively affected by EIV for nutrients and soil moisture, and negatively by herb layer cover (Table 2). Both at transect and plot level, EIV for nutrients explained the highest fraction of total deviance, followed by herb layer cover (Table 1, Table 2).

The number of *I. parviflora* individuals in spring at the transect level was positively affected by soil reaction, soil moisture and EIV for nutrients, and negatively by canopy openness and herb layer cover. Herb layer cover (Fig. 1) was the most important predictor of number of individuals in spring at the transect level as judged from the deviance explained by the different factors (Table 1). At the plot level, number of individuals in spring was significantly negatively affected by canopy openness, soil reaction and herb layer cover and positively affected by soil moisture (Fig. 1). Soil moisture and soil reaction explained most of the deviance in the data. There was also a significant interaction of year and soil moisture (Table 2). The effect of soil moisture was the strongest in 2012 and 2015 and weak or even not significant in the other years (data not shown).

Table 1: Optimal models for presence of *I. parviflora* and number of individuals in spring and in summer, juvenile mortality and number of fruits at the transect level selected based on AIC. Generalized mixed effect models with binomial (presence in spring and summer, juvenile mortality) or Poisson (number of individuals in spring and summer, number of fruits) error distribution. Not used – not relevant for the analyses, not included – not included in the optimal model. Significant results ($p < 0.05$) are in bold.

transect level analyses	presence in spring			number of individuals - spring			juvenile mortality			presence in summer			number of individuals - summer			number of fruits		
	deviance	p-value	effect	deviance	p-value	effect	deviance	p-value	effect	deviance	p-value	effect	deviance	p-value	effect	deviance	p-value	effect
year	**30.0**	**<0.001**		**246.2**	**<0.001**		**122.3**	**<0.001**		**39.1**	**<0.001**		**48.5**	**<0.001**		3.1	0.103	
number of colonized plots		not used		**5693.3**	**<0.001**	+		not used			not used		**5097.7**	**<0.001**	+		not used	
number of colonized plots previous year	**356.4**	**<0.001**	+		not used			not used		**399.2**	**<0.001**	+		not used			not used	
number of individuals previous year		not used		**2436.9**	**<0.001**	+		not used			not used		**2115.3**	**<0.001**	+		not used	
canopy openness	**22.1**	**<0.001**	-	**9.7**	**0.002**	-	**17.4**	**<0.001**	+		not included		**398.5**	**<0.001**	-	**54.2**	**<0.001**	-
EIV for nutrients	**217.1**	**<0.001**	+	**146.4**	**<0.001**	+	**304.6**	**<0.001**	-	**20.3**	**<0.001**	+	**28.2**	**<0.001**	+	**6.4**	**0.012**	+
soil moisture	**24.7**	**<0.001**	+	**52.8**	**<0.001**	+	**81.6**	**<0.001**	-		not included		**49.6**	**<0.001**	+	**13.7**	**<0.001**	+
soil reaction	**12.2**	**<0.001**	+	**73.9**	**<0.001**	+	**20.3**	**<0.001**	-	**4.4**	**0.036**	+	**15.0**	**<0.001**	+	**37.7**	**<0.001**	+
herb layer cover	**77.9**	**<0.001**	-	**1166.2**	**<0.001**	-		not included		**266.4**	**<0.001**	-	**625.6**	**<0.001**	-	**19.2**	**<0.001**	+

Table 2: Optimal models for presence of *I. parviflora* and number of individuals in spring and in summer, juvenile mortality and number of fruits at the plot level selected based on AIC. Generalized mixed effect models with binomial (presence in spring and summer, juvenile mortality) or Poisson (number of individuals in spring and summer, number of fruits) error distribution. Not tested – not relevant for the analysis, not included – not included in the optimal model. Asterisks indicate that the factors had significant effect when no other variables but covariates were included in the model. Factors that had no significant effect individually were not considered in the model at all. Significant results ($p < 0.05$) are in bold, marginally significant ($p < 0.1$) in italics.

plot level analyses	presence in spring			number of individuals - spring			juvenile mortality			presence in summer			number of individuals - summer			number of fruits		
	deviance	p-value	effect	deviance	p-value	effect	deviance	p-value	effect	deviance	p-value	effect	deviance	p-value	effect	deviance	p-value	effect
year	**84.4**	**<0.001** *		**9.9**	**0.019** *		**103.1**	**<0.001** *		**1.1**	**<0.001** *		**79.4**	**<0.001** *		**8.4**	**0.039** *	
distance from the path	**13.0**	**<0.001** *	-	**57.9**	**<0.001** *	-	**19.9**	**<0.001** *	+	**42.3**	**<0.001** *	-	**47.8**	**<0.001** *	-	**14.4**	**<0.001** *	-
presence in previous year	**90.8**	**<0.001** *	+	not used			not used			**88.7**	**<0.001** *	+	not used			not used		
number of individuals previous year	not used			**95.2**	**<0.001** *	+	not used			not used			**103.4**	**<0.001** *	+	not used		
canopy openness	not included *			**4.6**	**0.031** *	-	**9.5**	**0.002** *	+	2.1	0.1509 *		3.0	*0.083* *	-	**15.9**	**<0.001** *	-
EIV for nutrients	**110.2**	**<0.001** *	+	not included *			**17.3**	**0.004** *	-	not included			9.1	0.106 *		not included		
soil moisture	**30.5**	**<0.001** *	+	**53.4**	**<0.001** *	+	**44.8**	**<0.001** *	-	**72.2**	**<0.001** *	+	**32.0**	**<0.001** *	+	not included *		
soil reaction	not included			**36.6**	**<0.001** *	+	**56.6**	**<0.001** *	-	**29.2**	**<0.001** *	+	not included			not included		
herb layer cover	**41.5**	**<0.001** *	-	**7.1**	**0.008** *	-	0.3	0.558 *		not included			not included *			not included		
year:distance from the path	not included			**16.7**	**<0.001** *		**9.9**	**0.043** *		not included			not included			not included		
year:canopy openness	not included			not included			**32.9**	**<0.001** *		5.0	0.291 *		not included			not included		
year:EIV for nutrients	not included			not included			not included			not included			not included *			not included		
year:soil moisture	not included *			**14.3**	**0.003** *		not included *			5.8	0.216 *		**29.1**	**<0.001** *		not included		
year:soil reaction	not included			not included			not included			**10.9**	**0.027** *		**59.4**	**<0.001** *		not included		
year:herb layer cover	not included			not included			**41.3**	**<0.001** *		not included			not included			not included		

Juvenile mortality at the transect level was positively affected by canopy openness and negatively by EIV for nutrients, soil moisture and soil reaction. EIV for nutrients (Fig. 2) had the largest effect. Herb layer cover was not included in the optimal model (Table 1). At the plot level, juvenile mortality was positively affected by canopy openness and negatively by EIV for nutrients, soil moisture (Fig. 2) and soil reaction, with the last two factors explaining the highest fraction of total deviance. Significant interactions of year and canopy openness and of year and herb layer cover were also detected (Table 2).

Presence of *I. parviflora* in summer at the transect level was positively affected by EIV for nutrients and soil reaction and negatively by herb layer cover. Herb layer cover explained the highest fraction of total deviance (Table 1). At the plot level, presence of *I. parviflora* in summer was positively affected by soil moisture and soil reaction (Table 2).

Number of individuals in summer at the transect level was positively affected by soil reaction, soil moisture and EIV for nutrients and negatively by canopy openness and herb layer cover. Herb layer cover (Fig. 3) and canopy openness (Fig. 3) explained the highest fraction of total deviance (Table 1). At the plot level, number of individuals in summer was positively affected by soil moisture and by interaction of year and soil moisture, with the trends being the same as for number of individuals in spring. Additionally, there was a marginally significant negative effect of canopy openness and significant interaction of year and soil reaction (Table 2).

The number of fruits at the transect level was positively affected by EIV for nutrients, soil moisture, soil reaction and herb layer cover and negatively affected by canopy openness (Table 1). At the plot level, the number of fruits was negatively affected by canopy openness; no other factors except for the covariates had significant effect (Table 2).

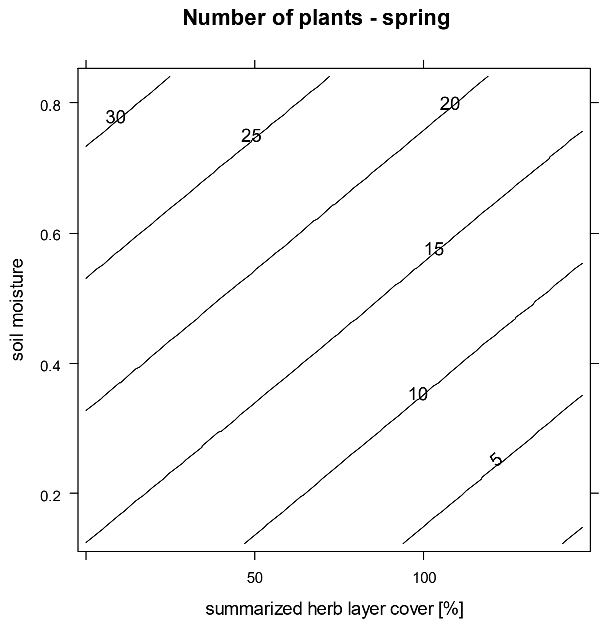

Fig. 1: Contour plot for number of *I. parviflora* plants in spring with summarized cover of herb layer and soil moisture as predictors.

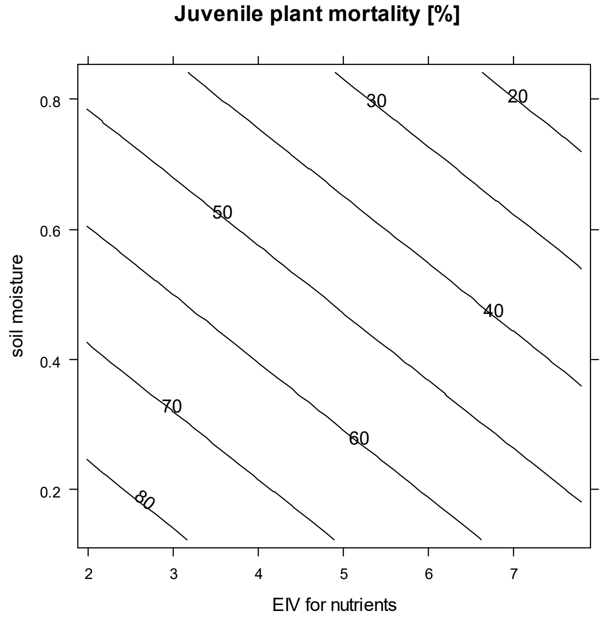

Fig. 2: Contour plot for juvenile mortality with EIV for nutrients and soil moisture as predictors.

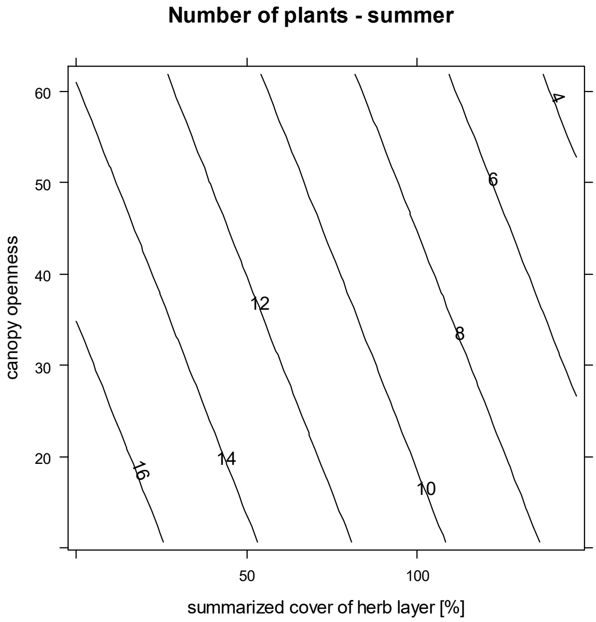

Fig. 3: Contour plots for number of *I. parviflora* individuals in summer with summarized cover of herb layer and canopy openness as predictors.

Discussion

Spread in different habitats

Impatiens parviflora spread the most into transects in oak-hornbeam forests, acidophilous oak forests and mixed coniferous forests. This is in line with other studies describing these habitats as optimal for *I. parviflora* (Pyšek et al. 2012, Reczyńska et al. 2015). *I. parviflora* spread also into transects in thermophilous oak forests and steppe grasslands on rocks, but it was not able to become dominant and form dense populations in these habitats. Further studies are required to see if it may represent any threat to these species rich communities by suppressing the native vegetation in the long term.

The spread of *I. parviflora* was mostly continual, but in summer 2012 we observed a decrease in the number of invaded plots in transects on steppe grasslands, and in termophilous oak forests compared to summer 2011. This decrease could be caused by exceptionally dry and hot weather in May and June in 2012 (based on data from Czech Hydrometeorological Institute, available from http://portal.chmi.cz/historicka-data) that lead to a high mortality of juveniles in the drier habitats. The decline in population size, however, did not affect the spread of *I. parviflora* in longer perspective and the populations fully recovered in the following year. Strong temporal variation in spread related to annual rainfall was previously reported for many other invasive species (e.g., Andrew and Ustin 2010, Bauer et al. 2002, Hobbs and Mooney 1991). The temporal variation points to the importance of longer term studies when studying habitat requirements of invasive species, since studies performed in one year can provide misleading information.

Factors affecting demographic rates of I. parviflora

Spread and performance of *I. parviflora* were affected by all studied environmental characteristics, i.e. canopy openness, soil moisture, soil reaction, nutrient availability and herb layer cover. However, the significance of individual environmental characteristics varied between individual demographic rates. It has been previously suggested that individual life cycle stages differ in their sensitivity towards environmental conditions and that conditions suitable for establishment do not need to be suitable for growth, survival and/or reproduction and vice versa (Milbau et al. 2003, Renne et al. 2006, Schupp 1995). It is therefore important to follow the whole plant life cycle when assessing habitat suitability, both when predicting species invasions and when searching for new habitats of rare or endangered species (Knappová et al. 2013, Turnbull et al. 2000).

Seedling emergence and establishment

The amount of nutrients was the most important predictor of the presence of *I. parviflora* seedlings, both at transect and plot level. The amount of nutrients was also the most influential predictor of juvenile mortality at the transect level. This is in line with Jarčuška et al. (2016), who depicted the level of nutrients as the most influential predictor of *I. parviflora* cover in temperate forests in the Western Carpathians, Slovakia. The negative effect of low nutrient levels on *I. parviflora* performance was highlighted also by Minden and Gorschlüter (2016), who experimentally compared its performance under nine combinations of light and nutrient availability. They found that the species is more prone to unfavorable nutrient conditions than to adverse light conditions. However, the species was also more plastic in response to nutrient level than to light intensity. The result is also supported by Reczyńska et al. (2015) studying the distribution of *I. parviflora* in oak forests in central Europe using phytocenological relevés. Even though they reported wide ecological tolerance of *I. parviflora* to all studied environmental characteristics, including nutrient levels, the species was most common in phytocoenoses with intermediate or high fertility, whereas it was absent or rarely recorded in vegetation typical of less fertile habitats.

Cover of herb layer was the most important environmental factor affecting the number of established seedlings at the transect level. This implies that transects with a high cover of herb layer and, thusly, a likely lower level of disturbance, are less threatened by *I. parviflora* invasion. Thus, a high cover of herb layer serves as a barrier against *I. parviflora* spread as was repeatedly suggested by other studies (e.g., Chmura and Sierka 2006, 2007, Klimko and Piskorz 2003, Obidzinski and Symonides 2000, Piskorz and Klimko 2007). Yet, *I. parviflora* sometimes also invades sites with a high cover of herb layer (Godefroid and Koedam 2010, present study). Our results show that high herb layer cover prevents seedling emergence and establishment, yet does not affect their mortality in later stages of the development. *I. parviflora* can therefore be found in habitats with high herb layer cover in case there was some disturbance in spring or the other species appeared later in spring than *I. parviflora* and gave *I. parviflora* seedlings enough time to establish. Varying effect of disturbances on seedling emergence and survival was previously shown for other invasive species such as *Lupinus polyphyllus* (Jauni and Ramula 2017) or *Rubus phoenicolasius* (Gorchov et al. 2011). Determining the effect of disturbances on individual stages of life cycle is important for risk assessment – species that require disturbances for seedling emergence and establishment, but not for survival, represent a greater threat. This is due to localized disturbances enabling these invaders to colonize the entire stand (Gorchoy et al. 2011).

At the plot level, soil moisture was the most important factor controlling the number of established seedlings. Soil moisture was also one of the most important factors affecting juvenile mortality, both at transect and plot levels. Its effect, however, largely varied between years. The effect was the strongest in years 2012 and 2015, which were exceptionally dry (based on data of the Czech Hydrometeorological Institute, available from http://portal.chmi.cz/historicka-data). Sufficient soil moisture was essential for seedling emergence and establishment, but the plants were able to tolerate much drier conditions in later stages of their development. Even though they wilted during the dry periods, they recovered quickly after rainfall. This finding can partially explain why *I. parviflora*, generally considered a meso-hygrophilous species, can under certain conditions grow in rather dry habitats (e.g., steppe grasslands on rocks in our study or xerophilous oak forests on rocky and sunny habitats in Reczyńska et al., 2015). The ability of adult *I. parviflora* plants to survive in very dry conditions was reported also by Quinet et al. (2015) demonstrating that *I. parviflora* plants were able to survive up to four weeks in soil moisture of less than 10 %, without any extreme loss of vigor. After the four weeks, reduction of photosynthetically active leaves was observed due to rapid senescence of older leaves under water stress.

Juvenile mortality

Juvenile mortality, at the transect level, was affected the most by EIV for nutrients, with higher survival rate on sites with higher nutrient content. The level of nutrients was also the most important predictor of presence of *I. parviflora* seedlings, meaning it plays a crucial role in all early stages of *I. parviflora* life. The effects of nutrients were more pronounced at the transect level compared to the plot level. This is most likely caused by a high variation in nutrient levels between transects and low variation within transects.

At the plot level, juvenile mortality was affected the most by soil reaction. *I. parviflora* performed worse in plots with acidic soil compared to plots with neutral or nearly neutral soil. Previous studies regarding *I. parviflora* requirements to soil reaction are ambiguous. According to Peace and Grubb (1982), the species is indifferent to soil reaction and grows on acidic, neutral and alkaline substrates. According to Chmura et al. (2007), the species is able to tolerate pH of 2.75 to 8.5, but prefers strongly acidic and neutral reaction over slightly acidic or alkaline. Similar result was reported by Reczyńska et al. (2015), in observing an increase in *I. parviflora* occurrence in acidophilous (from 3.0 to 4.2) and mesophilous communities (from 5.6 to 6.4) in oak hornbeam forests of central Europe. Contrary to this, Lawesson (2003) suggests that *I. parviflora* can be used as an indicator of acidic soils in Denmark with an optimum soil reaction of 4.4. It is possible that the species has different requirements for soil reaction in different geographical areas, which is true for many other species as well (see Schaffers and Sýkora (2000) and references therein).

Presence and number of I. parviflora individuals in summer

Both presence of *I. parviflora* and number of individuals in summer was affected the most by cover of herb layer at the transect level and by soil moisture at the plot level. Presence and number of individuals in summer is a combination of the two previously described processes, seedling emergence and establishment and juvenile mortality. Since the most important factors affecting presence and number of individuals in summer are the same as the factors affecting seedling emergence and establishment, we can conclude that the early stages of *I. parviflora* life play the key role in its invasion success. Number of individuals in summer was also largely affected by canopy openness, which was the most important factor affecting number of fruits, pointing to the importance of sufficient seed availability.

Fitness

The most important factor affecting *I. parviflora* fitness (expressed as number of fruits produced) was canopy openness, both at transect and plot levels. Canopy openness had overall negative effect on *I. parviflora* performance and the plants produced fewer fruits under open canopy. The negative effect of direct sun radiation on *I. parviflora* growth has been known for a long time. Under intense direct radiation, the plants wilt even when water supply is not limiting (Coombe 1956). On places with open canopy, *I. parviflora* plants are less likely to survive till maturity, due to their being smaller and producing fewer fruits than in more shaded places. Suggested optimum for *I. parviflora* is a canopy openness of 30-40 %, which corresponds to open forests or gaps between trees (Piskorz 2005, Reczyńska et al. 2015).

Conclusions

In this study, we observed the natural spread of *I. parviflora* into new habitats. We showed that *I. parviflora* can rapidly spread within oak-hornbeam, acidophilous oak, and mixed coniferous forests that represented its optimum habitat in the studied area. It also spread into thermophilous oak forests and into steppe grasslands on rocks, even though it only formed loose populations in these habitats. Contrary to the other habitats, these two are species rich habitats, often hosting endangered plant species. Even though the species has low abundance in these habitats and may thus not pose any serious threat to these communities, we recommend this to be tested and further spread to be monitored.

We showed that individual environmental conditions differ in their importance for individual stages of *I. parviflora* life. Herb layer cover effectively prevented seedling emergence and establishment, but its effect on older plants was limited. Thus, the plant can grow in sites with high cover of herb layer in case the other species in herb layer emerged later in spring or summer and gave *I. parviflora* seedlings enough time to establish. Sufficient soil moisture was also important for seedling establishment and survival, but had weaker effect on adult plants. In years with sufficiently moist springs that allowed seedlings to establish, *I. parviflora* survived in dry habitats such as steppe grasslands on rocks, even though it had lower fitness and was favoring moister microhabitats. High levels of nutrients were improving *I. parviflora* performance and were mainly important for seedling emergence and establishment and for juvenile survival. Under low nutrient levels, seedlings had a much lower chance to establish and had a higher mortality rate, resulting in lower summer abundance of the species. Canopy openness had a negative effect on *I. parviflora* performance. *I. parviflora* performed better in neutral soils compared to acidic soils.

Overall this study illustrates that monitoring natural spread of invasive species can provide important insights into the determinants of their distribution. It also illustrates that the determinants depend on spatial scale studied and differ between different life stages of the species.

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Appendix

Appendix 1: *Impatiens parviflora* occurrence in individual transects. Total length shows number of plots in a transect, subsequent columns show number of plots invaded by *I. parviflora* in given time.

transect	total length	summer 2011	spring 2012	summer 2012	spring 2013	summer 2013	spring 2014	summer 2014	spring 2015	summer 2015	spring 2016	summer 2016
acidophilous oak forest	44	26	39	26	41	31	44	33	39	30	44	37
heathland, former pasture 1	23	3	2	0	1	1	3	1	2	1	4	2
heathland, former pasture 2	23	3	2	2	2	2	4	3	2	1	5	2
heathland, former pasture 3	17	4	4	0	0	0	1	0	0	0	3	1
heathland, former pasture 4	33	3	3	0	0	0	0	0	0	0	2	0
mixed coniferous forest 1	15	5	15	14	15	15	15	15	14	14	15	15
mixed coniferous forest 2	15	4	15	13	15	15	15	15	14	14	15	15
oak-hornbeam forest 1	25	10	23	18	25	25	25	25	23	22	25	25
oak-hornbeam forest 2	14	3	12	6	14	14	14	14	13	12	14	14
steppe grassland on rocks 1	29	11	3	1	17	15	27	16	22	15	26	18
steppe grassland on rocks 2	14	3	5	4	12	8	14	8	14	8	14	9
steppe grassland on rocks 3	18	6	3	0	18	16	18	17	16	15	18	17
thermophilous oak forest 1	16	5	11	0	4	6	13	7	12	6	14	9
thermophilous oak forest 2	18	7	11	4	14	14	18	16	16	14	18	17
thermophilous oak forest 3	17	7	11	4	12	13	16	15	15	14	17	15
total	321	100	159	92	190	175	227	185	202	166	234	196

Appendix 2: Correlation matrix of explanatory variables. Significant values ($p < 0.05$) are in bold.

	canopy openness	EIV contin.	EIV light	EIV moisture	EIV nutrients	EIV soil reaction	EIV temp.	graminoid cover	herb layer cover	soil moisture	soil reaction	species number
canopy openness	1											
EIV - continentality	0.198	1										
EIV - light	0.404	-0.042	1									
EIV - moisture	-0.366	-0.149	**-0.552**	1								
EIV - nutrients	-0.136	0.144	-0.022	0.116	1							
EIV - soil reaction	0.241	0.239	0.240	-0.216	**0.622**	1						
EIV - temperature	0.137	**0.560**	0.008	-0.292	0.051	-0.026	1					
graminoid cover	0.505	0.160	0.280	-0.254	-0.308	0.060	0.218	1				
herb layer cover	0.392	-0.046	0.354	-0.243	**-0.502**	-0.097	0.003	**0.726**	1			
soil moisture	**-0.594**	-0.277	**-0.576**	**0.546**	0.024	-0.243	-0.262	**-0.529**	-0.295	1		
soil reaction	0.289	0.128	0.160	-0.052	0.345	**0.735**	-0.119	0.268	0.110	-0.229	1	
species number	0.406	-0.118	0.374	-0.300	-0.482	-0.089	-0.028	**0.544**	**0.837**	-0.272	0.060	1

Appendix 3

See Fig. 4

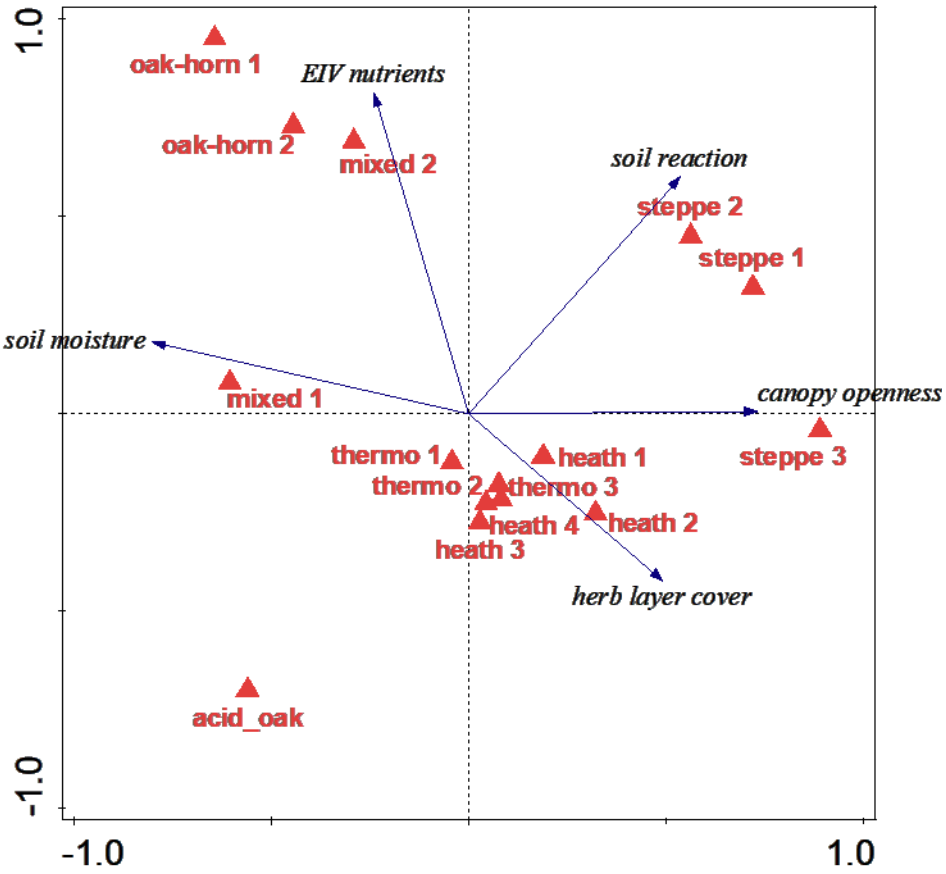
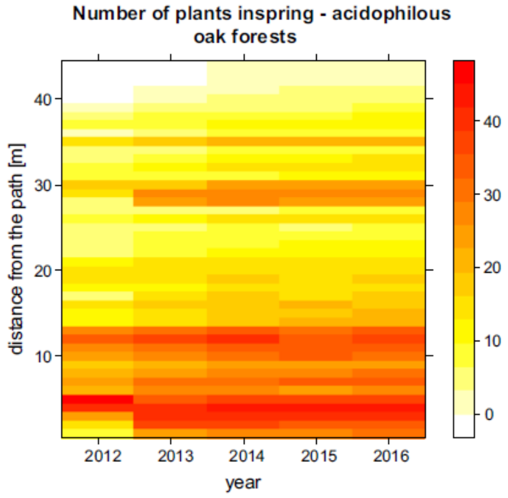


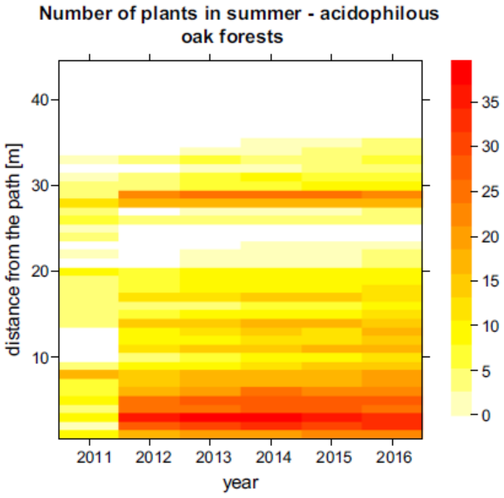
Fig. 4: Relationship of individual transects with environmental characteristics used in statistical analyses. Ordination plot RDA. Dependent variables – standardized environmental characteristics, independent variable – transect (15 points). The model explained 70 % of variability in the data. 499 permutations, p = 0.002. acid_oak – acidophilous oak forest, heath – heathland on former pasture, oak-horn – oak-hornbeam forest, mixed – mixed coniferous forest, steppe – steppe grassland on rocks, thermo – thermophilous oak forest, numbers refer to individual transects (see Appendix 1).

Appendix 4

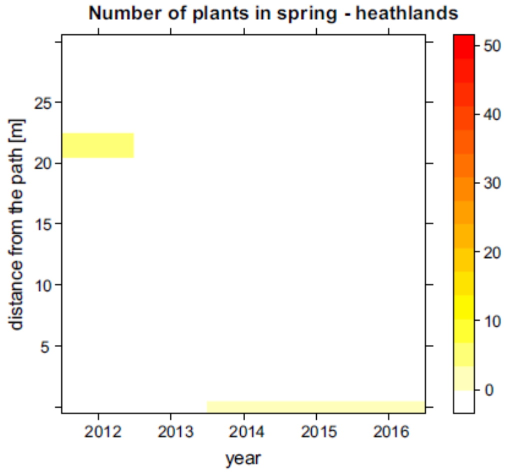
See Fig. 5



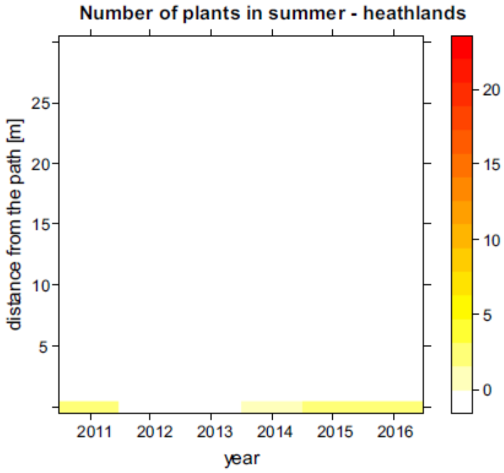
Number of plants inspring - acidophilous oak forests



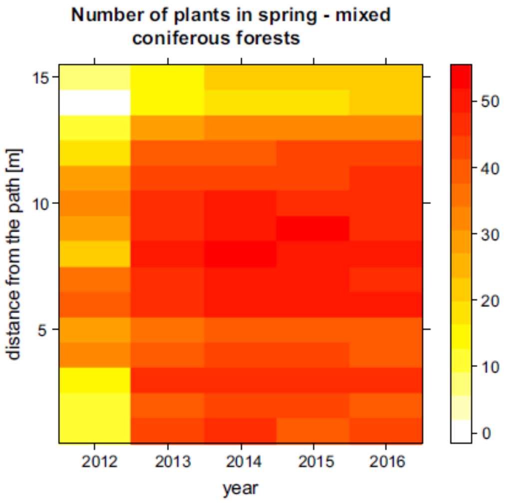
Number of plants in summer - acidophilous oak forests



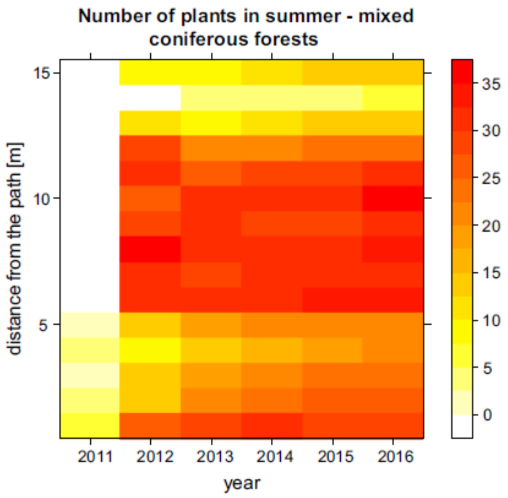
Number of plants in spring - heathlands



Number of plants in summer - heathlands



Number of plants in spring - mixed coniferous forests



Number of plants in summer - mixed coniferous forests

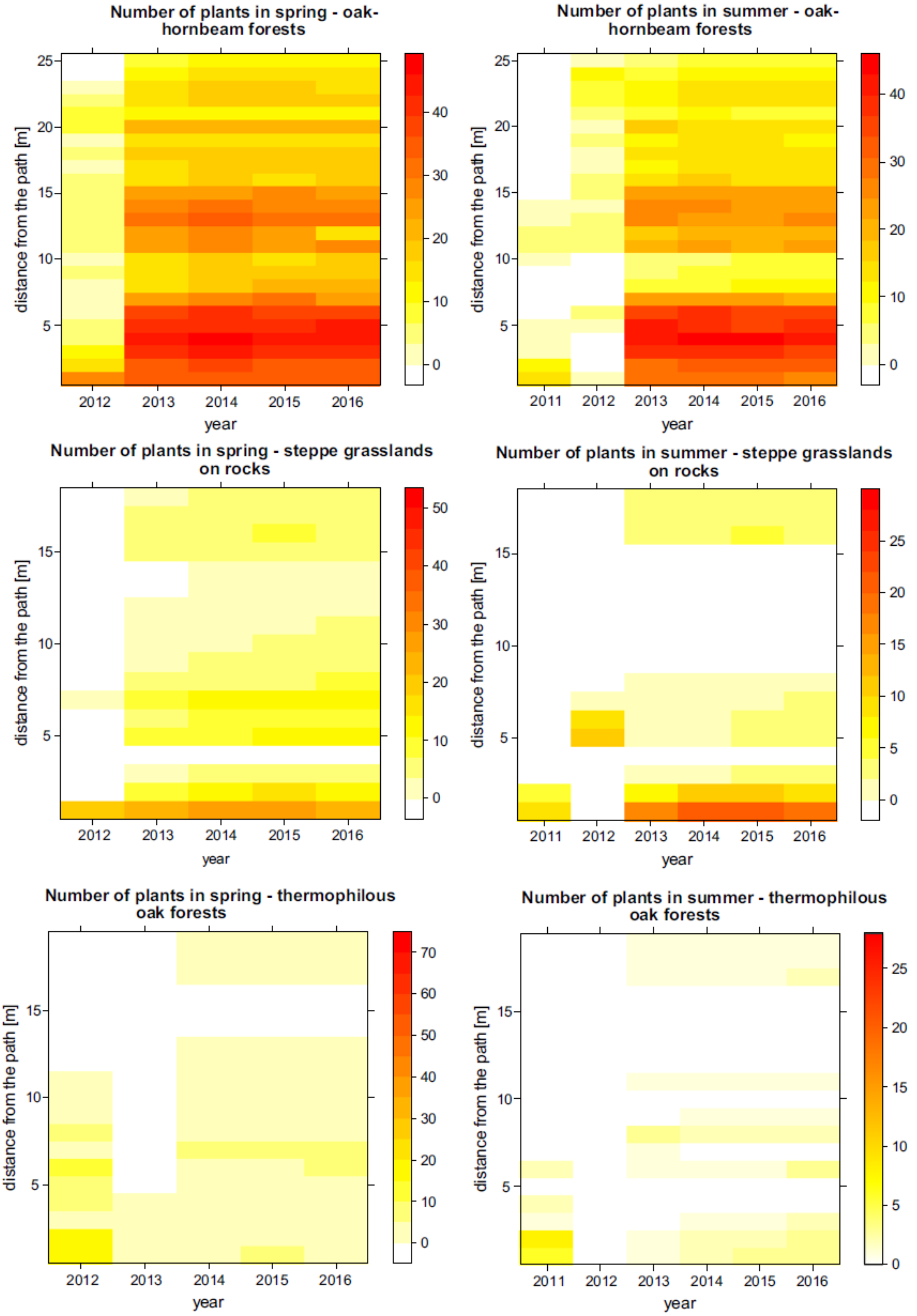


Fig. 5: Number of *I. parviflora* individuals in spring and summer in individual types of habitats in relation to distance from the path (i.e. source population) and year of sampling. All transects from given habitat type are used in each figure.

Study 5

Invasive *Impatiens parviflora* has negative impact on native vegetation in oak-hornbeam forests

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Abstract

Impatiens parviflora (Balsaminaceae) is one of the most widespread invasive plant species in Central Europe. Nevertheless, both mechanisms and consequences of its invasion are still poorly understood. In this study we attempt to understand the impact of this species on native vegetation.

The impact of *I. parviflora* on native vegetation was studied using removal experiment on permanent plots in oak-hornbeam forests in central Bohemia, Czech Republic. Nine pairs of plots were established in invaded vegetation, one plot in each pair stayed invaded and the other served as removal plot and all *I. parviflora* individuals were repeatedly removed from the plot. Species composition in 4 subsequent years was recorded in the plots. Species response to *I. parviflora* removal was correlated to plant traits to reveal trait characteristics of species suppressed by the invasion.

Significant increase in both numbers and cover of native species was observed in removal plots in comparison with invaded plots during the experiment, with the greatest change in first two years after invader removal. Species composition also significantly differed between invaded and removal plots. Species with high affinity to removal plots, i.e. species that are most restricted by *I. parviflora* invasion, were mostly species with small releasing height and early start of flowering.

Our results indicate that *I. parviflora* has negative impact on native vegetation but that the vegetation can recover within few years after the invader removal. Due to its extensive distribution and high turnover, removal of the species from larger plots is, however, not realistic in practice.

Key Words: forest understory herb; deciduous forest; plant functional trait; removal experiment; small balsam; vegetation recovery.

Introduction

Biological invasions are a key topic of ecological research in the last decades (Sod 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, Worz and Thiv, 2015), undermine functioning of whole ecosystems (Richardson and Pyšek, 2012) and cause significant economic losses (Zavaleta, 2000). While the increasing number and expanding ranges of non-native species in the world flora are very well documented (Lonsdale, 1999; Pyšek and Hulme, 2005), their impacts remain unquantified for most alien plants (Barney et al., 2013; Pyšek et al., 2012). Moreover, ecological impacts of most invasive plants have not been studied experimentally (Barney et al., 2015) and our knowledge of their impact comes from observational studies that have compared invaded and non-invaded habitats (Levine et al., 2003). However, as species composition and diversity may themselves influence the likelihood of invasion, separating cause and effect using such correlative approach is nearly impossible (Levine and D`Antonio, 1999). Alternative approaches include either experimental introduction (e.g., Flory and Clay, 2010; Maron and Marler, 2008) or removal of the invasive species (Guido and Pillar, 2015; Kumschick et al., 2015). As experimental introductions of invasive species into natural or semi-natural habitats are understandably not encouraged, removal experiments seem to be the best way to study the impact of invasive species on native vegetation (Zavaleta et al., 2001).

Several removal experiments have already been performed to assess the impact of invasive species such as *Impatiens glandulifera* (Hejda and Pyšek, 2006; Hulme and Bremner, 2006), *Alliaria petiolata* (Stinson et al., 2007) or *Mimulus guttatus* (Truscott et al., 2008) on native vegetation. For most species, comparative studies still remain the main source of information (for complete list of invasive species for which removal experiments were done see Guido and Pillar (2015)). One of the species that lacks good data from removal experiments is *Impatiens parviflora,* an invasive plant of Central and East Asiatic origin that belongs to most widespread invasive plants in Europe. (Lambdon et al., 2008).

As this alien is often seen dominating herbal layer of invaded forests, including sites with relatively low hemeroby, it can potentially impact rare native species (Godefroid and Koedam, 2010). However, its interaction with native species of invaded communities is surprisingly poorly documented (Godefroid and Koedam, 2010). Negative impact of *I. parviflora* has been reported by a few studies (Chmura and Sierka, 2006; Dobravolskaitė, 2012; Łysik, 2008; Obidzinski and Symonides, 2000). However, because of the comparative approach of the studies, it is not clear, whether the changes in the vegetation of herbal layers are caused by the invasion of *I. parviflora* or if *I. parviflora* just profits from changes caused by another factors. Hejda (2012), who as the only one aimed to disentangle these two options by using removal experiment, reported only minuscule, if any, impact of the species on native vegetation. Specifically, he observed no significant changes in cover of native species and only minor differences in species composition in removal and invaded plots. However, Hejda (2012) in his study compared large set of very variable removal and invaded plots, instead of performing more precise pair-wise experiment. Also the impact of the species could be weakened due to rather late removal of the invader. As seedlings of *I. parviflora* grow fast, they can possibly suppress other species already in the first few weeks after germination. Therefore, it seems more appropriate to set up the plots before the season and start removing *I. parviflora* seedlings immediately after germination to avoid any possible impact on native vegetation. Lastly, Hejda`s (2012) study took only two years which might not be enough for all changes to take place. For the above mentioned reasons, another removal experiment for *I. parviflora* should be done, with following differences: i) perform pair-wise comparisons of plots, ii) remove *I. parviflora* seedlings immediately after germination, iii) run the experiment for longer than two seasons only.

It has been suggested that *I. parviflora* cannot have strong impact on native vegetation as it is competitively rather weak (Čuda et al., 2015). As an annual species with modest root system (Slavík, 1997), it is unlikely that it will compete with native plants via belowground competition. Aboveground competition is much more likely, given that *I. parviflora* is locally very abundant and often creates dense stands on small spatial scales (Dostál et al., 2012) and therefore changes the light conditions for understory species (Uherčíková and Eliáš, 1987). However, *I. parviflora* usually competes with forest herbs and tree seedlings which are well adapted to low light condition (Hejda, 2012). Therefore, we think that intensive competition during the early phases of the season might be the only mechanism how *I. parviflora* impacts native vegetation. To test this hypothesis, we correlated selected species traits with the level of their suppression by *I. parviflora* invasion. If mostly early spring, heliophilous or small plants get suppressed by the invasion, it will indicate competition for light. If the level of suppression is correlated with other plant traits, such as nutrient or water supply requirements, below-ground competition will be more likely.

In this paper we aim to assess the impact of *I. parviflora* on native vegetation by comparing heavily invaded vegetation with dominant *I. parviflora* and heavily invaded plots from which

I. parviflora had been removed. If the vegetation on removal plots develops in a different way compared to the invaded plots (i.e. interaction of treatment and year will significantly affect the species composition, number or cover of native species), it will suggest that *I. parviflora* affects native diversity or composition of the invaded communities. In this case, *I. parviflora* would be the driver of the changes associated with the invasion. However, if the vegetation on removal and invaded plots is the same at the end of the experiment, it will show that *I. parviflora* itself does not affect the vegetation and more likely acts as a passenger of the environmental changes associated with the invasion. The overall aim of this study is to answer the following questions: i) What is the effect of invader removal on species composition, number and cover of native species? ii) What are the traits of plants most suppressed by *I. parviflora* invasion?

Material and Methods

Study Species and Area

Impatiens parviflora is an annual representative of Balsaminaceae family, native in Eastern and Central Asia. The species was first introduced to Europe in 1831 as an ornamental plant (Coombe, 1956). Since then it spread through whole Europe, except for the very south and north of the continent (Trepl, 1984). It has been reported from 34 European countries in total (Lambdon et al., 2008), with Central Europe being one of the most widely invaded area. In the Czech Republic, it was first introduced in 1844, it became naturalized already in 1870s (Slavík, 1997). The species is common over the whole area of the Czech Republic except for very dry and mountain areas (Pyšek et al., 2012b). Specific characters of *I. parviflora* such as short life cycle, presence of cleistogamic and chasmogamic flowers, high production of seeds, long duration of flowering, fast growth of seedlings and high tolerance to light conditions (Coombe, 1956; Eliáš, 1999; Perrins et al., 1993,) make it one of the most ideal weeds (Noble, 1989) able to colonize high number of habitats. In the Czech Republic, it has been reported from 45 habitat types and it is considered a dominant species in number of them, including perennial nitrophilous herbaceous vegetation of mesic sites, herb layers of alluvial forest, oak-hornbeam forests, ravine forests and *Robinia pseudoacacia* plantations (Pyšek et al., 2012b; Sádlo et al., 2007).

The study was carried out in oak-hornbeam forests near nature reserve Zvolská Homole, central Bohemia, Czech Republic (49°56'24''N, 14°24'09''E). Oak-hornbeam forests are one of the most typical stands for *I. parviflora*. The particular area was chosen because it is massively invaded, which made it suitable for removal experiments. Also it is easily accessible, and could thus be visited weekly during the whole vegetation season.

Experimental Design

Nine pairs of 1 × 1 meter permanent plots were established in August 2011 in homogenous part of the forest invaded by *I. parviflora*. The two plots of one pair were established in the same distance from a path (representing the intensity of trampling by tourists and dogs), on the same inclination, they had the same canopy openness, level of *I. parviflora* invasion (approximately 50% coverage) and as similar vegetation as possible. One plot in each pair stayed invaded (hereafter referred to as invaded plot). All *I. parviflora* plants were removed from the other one, hereafter referred to as removal plot, in August 2011. Subsequently, *I. parviflora* seedlings were removed from the removal plots every week in vegetation season in years 2012-2015.

Small scale plots (1 × 1 m) were chosen, as it would not have been possible to eradicate *I. parviflora* from larger plots without inducing severe disturbance to the removal plots. Given the low density of the understory, most plots harbored just 3-7 species with some of the plots actually being empty in summer and not harboring any native species (these two plots remained empty during the whole experiment, even after *I. parviflora* removal, indicating very unfavourable conditions). Vegetation on such small scale plots is prone to be influenced by edge effects. Removal plots could have been impacted by the surrounding stands of *I. parviflora*. Conversely, the vegetation on invaded plots could have been influenced by the *I. parviflora* being trampled in the surroundings when recording the vegetation data. To minimize the edge effects on the removal plots, *I. parviflora* was removed also from the 0.5 m belt around the removal plots. On the contrary, to minimize the edge effects on the invaded plots with intact stands of *I. parviflora*, there was a 0.5 m belt around the invaded plot that also stayed invaded and plots were inspected and data collected from a distance to leave at least 0.2 m wide belt of intact vegetation with *I. parviflora* around the invaded plots. The two plots of a pair were always exactly 1 m apart, touching by the 0.5 m wide belts around each plot. The distance between the pairs varied from 10 to 20 m.

Each plot was further divided into 9 subplots. Phytocenological reléves were recorded on each subplot twice a year, in late April (referred to as spring) and early June (referred to as summer), in 2012-2015. All vascular plant species and their cover were recorded. The cover was estimated to units of percent at plants with less than 10 % cover (i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 %)

and to tens of percent at plants with higher cover (i.e. 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 %). Mosses and lichens were not determined, only their total cover was recorded. Total cover of herbaceous layer was calculated as a sum of covers of all species in the herbaceous layer except for *I. parviflora*, number of species was determined as the number of all species in a subplot except for *I. parviflora*. *I. parviflora* was excluded from the species-sample matrix before data analyses as the manipulated species of interest should not be an integrated part of the measured response and as it is excluded in most manipulative experiments (Thomsen et al., 2016). The information on its cover is, however, provided in Supplement 1.

Statistical Analyses

As understory vegetation of oak-hornbeam forests differs a lot between spring and summer season due to very strong spring aspect (different species, different total cover etc.), all analyses were performed separately on datasets from spring and from summer.

Changes in numbers of species and species covers of herbaceous layer in removal vs. invaded plots were tested with linear mixed effect models. Number of species in a subplot and square root transformed species cover were used as dependent variables, independent variables were treatment (invaded vs. removal plot), year and their interaction. Code of each plot and code of each subplot served as random effects. F-tests comparing two models with and without a tested term were used to estimate p-values.

Possible differences in species composition following the removal of *I. parviflora* were tested using linear direct gradient analysis (Redundancy Analysis, RDA) and Monte–Carlo permutation tests (ter Braak and Šmilauer, 1998) with 499 permutations (plots were permutated only within the block). Before this, an indirect model (Detrended Corespondence Analysis, DCA) was used to decide whether to use a linear or a unimodal approximation (ter Braak and Šmilauer, 1998). The interaction term between treatments and time was used to test possible changes in plots following the removal of *I. parviflora*. Square root transformed cover of herbaceous layer were used as dependent variables, year, treatment and block (each pair of 1 × 1 m plots form one block, 9 blocks in total) were set as covariables, while the interactions between time and treatment were predictor variables. Time was considered a continuous variable in the ordination models, as recommended by Lepš and Šmilauer (2000). To compare the relative importance of treatment, year and their interactions, the same analysis was performed with treatment and year respectively as predictor variables; the other variable always served as a covariable.

The relationships between species scores from the first canonical axis from RDA (interaction of treatment and year as predictor variables) and selected plant traits were tested to reveal general characteristics of plants suppressed by the invasion. Selected plant traits included life span (annual vs. perennial), life form (phanerophyte, geophyte, terophyte, hemicryptophyte), specific leaf area (hereinafter referred to as SLA), releasing height, month of start of flowering, month of the end of flowering and Ellenberg indicator values (hereinafter as EIV) for nutrients, soil reaction, moisture, light and temperature. For SLA, life span and life form, scores of all species from RDA were used for the analysis. For month of start and the end of flowering, releasing height and EIV, tree seedlings scores were excluded from the analyses. Data for flowering and fruiting are not relevant for tree seedlings, EIV are available only for adult trees that commonly differ in habitat requirements from their seedlings. Data on life span, life form, SLA and releasing height were obtained from LEDA database (Kleyer et al., 2008), data on start and end of flowering were taken from Kubát et al. (2002). The dependence of species scores on plant traits was tested with linear models at numeric variables and with ANOVA at categorical variables.

All univariate analyses were performed in R.3.1.0 (R Development Core Team, 2014). All multivariate analyses were performed in Canoco 5 (ter Braak and Šmilauer, 2012).

Results

In total, 20 herb species and seedlings of 5 tree species were found in herbal layer of all plots (see Supplement 1 for details). The number of native species in a subplot (33 × 33 cm) varied from 0 to 11, with 3 on average. Total cover of native species (sum of covers of all species) varied from 0 to 255 %, with average of 44 %. Number and cover of native species were highly correlated (in spring $r = 0.85$, in summer $r = 0.82$), therefore only results for number of native species are presented in the text. Results for cover of native species are shown in Supplement 2.

Effect of interaction of treatment and year on number of species was significant both in spring and summer datasets. The number of species was slightly increasing in removal plots in time, while the number in invaded plots stayed approximately the same (Fig. 1). In both spring and summer dataset, the number of species was significantly higher in removal than in invaded plots

(Fig. 1). Year effect was significant in both datasets as well, with significantly lowest number of species in 2012 (Table 1).

Table 1: Dependence of number of species and species composition on treatment, year and treatment × year interaction. Df Error = 56. Significant results are in bold.

		number of species				species compostion					
		spring		summer		spring			summer		
	df	p	F	p	F	p	pseudo-F	% var.	p	pseudo-F	% var.
treatment	1	**<0.001**	**20.80**	**0.002**	**11.37**	**0.002**	**130.0**	**17.0**	**0.002**	**57.30**	**8.3**
year	3	**0.018**	**18.24**	**<0.001**	**33.83**	**0.004**	**13.1**	**2.0**	**0.002**	**20.80**	**3.2**
treatment×year	3	**<0.001**	**73.37**	**<0.001**	**11.90**	**0.002**	**34.7**	**5.2**	**0.004**	**19.00**	**2.9**

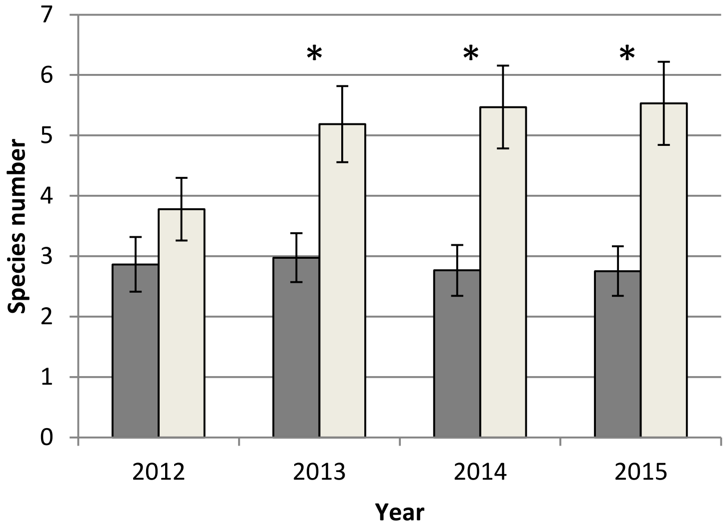


Fig. 1: Number of native species in invaded and removal plots in spring in each of four sampling years (mean ± SE). Significant differences between removal and invaded plots indicated by an asterisk (*).

Effect of interaction of treatment and year on species composition (Fig. 2) was significant both in spring and summer datasets. The model for spring explained 5.2 % variability in the data (variability explained by the first canonical axis in the indirect analysis, PCA, i.e. possible maximum of variability explained by one canonical axis, was 15.6 %). The model for summer explained 2.9 % of the variability in the data (PCA explained 18.1 %). Both effect of treatment and year were significant in both spring and summer datasets, treatment explained the most variability in the data in both cases (Table 1).

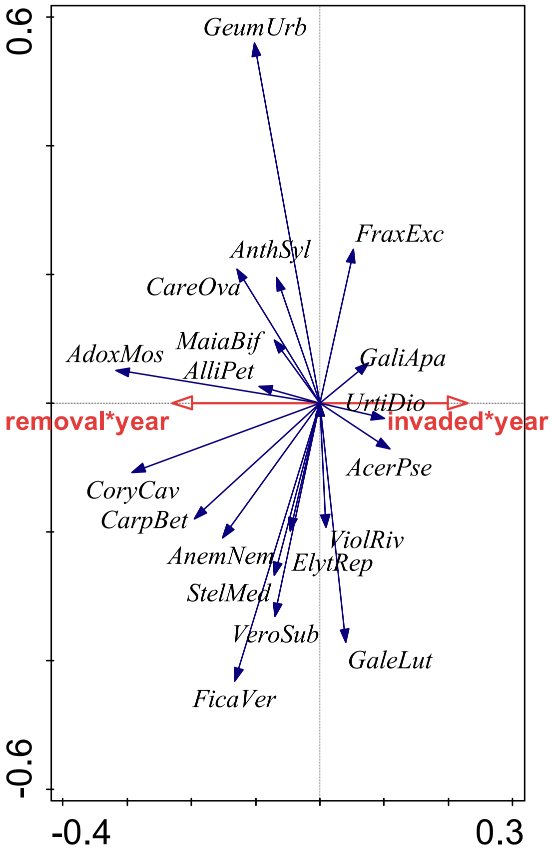
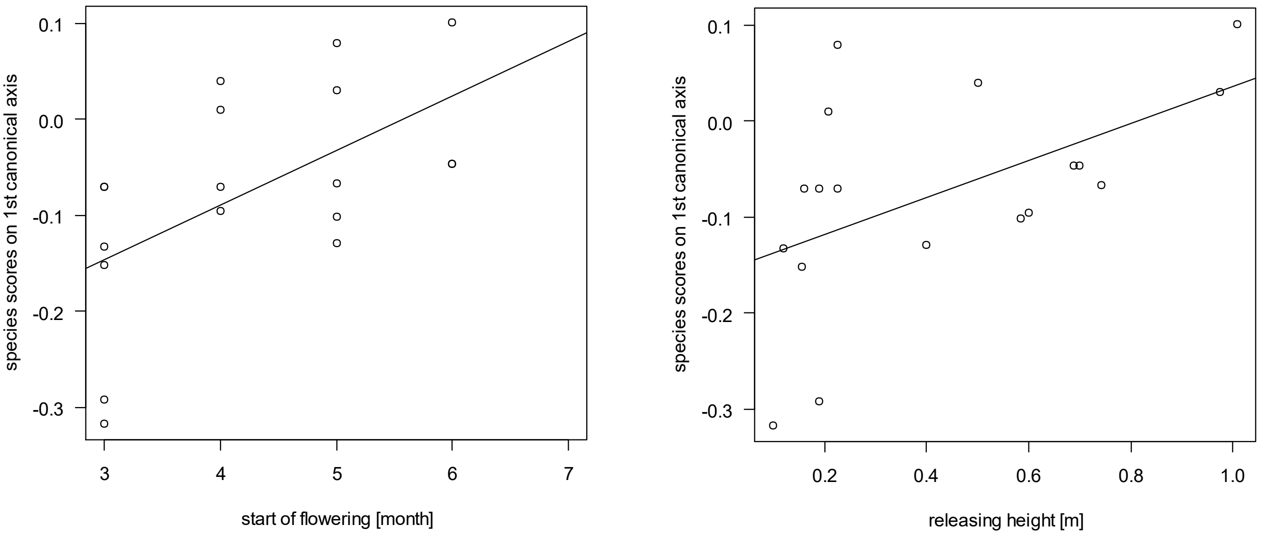


Fig. 2: Ordination plot of a model testing the changes in spring species composition in time in plots with different treatments. The species' percentage cover estimates were used as dependent variables. RDA, 499 permutations, p = 0.002, first canonical axis explains 5.2 % of the variability of the data. Species names are abbreviated: AcerPse = *Acer pseudoplatanus*, AdoxMos = *Adoxa moschatellina*, AnemNem = *Anemone nemorosa*, CareOva = *Carex ovalis*, CarpBet = *Carpinus betulus*, CoryCav = *Corydalis cava*, ElytRep = *Elytrigia repens*, EuonEur = *Euonymus europaeus*, FicaVer = *Ficaria verna*, FraxExc = *Fraxinus excelsior*, GaleSpe = *Galeopsis speciosa*, GaleLut = *Galeobdolon luteum*, GaliApa = *Galium aparine*, GeraRob = *Geranium robertianum*, GeumUrb = *Geum urbanum*, MaiaBif = *Maianthemum bifolium*, PoaNem = *Poa nemoralis*, QuerRob = *Quercus robur*, StelMed = *Stellaria media*, SympTub = *Symphytum tuberosum*, UrtiDio = *Urtica dioica*, VeroSub = *Veronica sublobata*, ViolRiv = *Viola riviniana.*

Species scores from spring model were significantly correlated with the month of start of flowering (p = 0.011, $R^2 = 33.9$ %, Fig. 3a) – early spring species occur more often in removal plots, while late flowering species occur more often in invaded plots. Species scores from spring model were further significantly correlated with releasing height (p = 0.025, $R^2 = 26.2$ %, Fig. 3b) – species that are increasing in number in removal plots in time are usually these with small releasing height, while plants occurring more often in invaded plots are these with higher releasing height. Effect of SLA on species scores was also significant (p = 0.002, $R^2 = 40.0$ %), but the dependency was determined only by three species with high SLA (*Adoxa moschatellina*, *Corydalis cava* and *Poa nemoralis*), so the results must be interpreted with caution. Effects of EIV, month of the end of flowering, life form and length of life on species scores from spring model were not significant (F < 1.92, p > 0.181).



a) b)

Fig. 3: Significant linear relationships between RDA species scores on the first canonical axis (see Fig. 2) and a) month of start of flowering, b) releasing height. Negative numbers – removal plots × year, positive numbers – invaded plots × year.

Species scores from summer model were significantly correlated with the month of the end of flowering (p = 0.014, R^2 = 36.3 %) – species coexisting with *I. parviflora* in invaded plots are flowering till late summer or autumn, while species that increase in number after *I. parviflora* removal are flowering in spring or early summer. Effects of EIV, month of the start of flowering, releasing height, SLA, life form and length of life on species scores from summer model were not significant (F < 4.53, p > 0.052).

Discussion

The study demonstrated that *I. parviflora* has negative impact on native vegetation. As both number and cover of species increased after *I. parviflora* removal, *I. parviflora* seems to be the driver, not the passenger, of the changes associated with the invasion. Negative impact of the species was previously indicated by other studies using different methodological approaches (e.g., comparing invaded and non-invaded vegetation (Dobravolskaitė, 2012), comparing vegetation before and after invasion (Łysik, 2008), correlating native species richness and cover with cover of *I. parviflora* (Obidzinski and Symonides, 2000; Jarčuška et al., 2016)).However, results of these studies might be interpreted both as impact of *I. parviflora* on native vegetation and as effect of native vegetation on the spread of *I. parviflora*. Hejda (2012), who as the only one used removal experiment to study the impact of *I. parviflora* on native vegetation, observed some changes in species composition and cover, however, these were not significant (see Introduction for details).

Our results are in line with the hypothesis that the impact of *I. parviflora* is mostly due to above-ground competition for light for the following three reasons. i) The changes in vegetation were much more pronounced in spring dataset than in summer data set. ii) Species that are most affected by *I. parviflora* invasion, i.e. species that increased their cover after *I. parviflora* removal, are mostly early flowering species with small releasing height. These species, including heliophilous early spring species such as *Adoxa moschatellina*, *Anemone nemorosa*, *Corydalis cava* or *Maianthemum bifolium,* are not adapted to low light availability and therefore are vulnerable to any aboveground competition, even though the competitor is rather weak. iii) *Impatiens parviflora* seems to cause no long-term changes to the community and the vegetation recovers quite quickly after its removal. If the invader changed the level of available nutrients or released some allelopathic chemicals, recovery of the vegetation would take much longer (Corbin and D`Antonio, 2012; Grove et al., 2015; Lankau et al., 2014). These results are in agreement with the results of studies on closely related species *Impatiens glandulifera* that also reported fast regeneration of the vegetation after invader removal (Hejda and Pyšek, 2006; Hulme and Bremner, 2006).

Due to differences in methodology, it is not possible to compare quantitatively the impact of *I. parviflora* with impacts of other invasive species. The impact of *I. parviflora* is not as dramatic as the impact of species such as *Heracleum mantegazzianum* or *Reynoutria* species that can reduce species richness of invaded community up to 90 % compared to non-invaded vegetation (Hejda et al., 2009). *I. parviflora* mostly changes the cover of native species, not their presence/absence, which makes it similar to its relative *I. glandulifera* (Hejda and Pyšek, 2006). *I. glandulifera*, much more robust plant colonizing mostly river banks, has been considered a danger for native vegetation and has been listed in the list of 100 worst invasive species of the world (DAISIE, 2009). It, however, seems that its impact causing approximately 25% decrease in species richness (Hejda et al., 2009; Hulme and Bremner, 2006) is not more serious than the impact of the rather subtle *I. parviflora*, generally considered harmless. What makes these two species similar is their annual character and shallow and modest root system that does not allow them to compete effectively for water and therefore limits their impact to above-ground competition. Another factor affecting the rather low impact of both *Impatiens* species is that they often grow in habitats with native dominants of similar physiognomy and impact to the surrounding vegetation (for *I. glandulifera* it is high nitrophilous vegetation, for *I. parviflora* for example similar closely related native *I. noli-tangere*) (Hejda et al., 2009). However, contrary to *I. noli-tangere*, *I. parviflora* germinates earlier and its seedlings grow faster (Perglová et al., 2009), so it is more likely to affect the spring vegetation than the native dominant.

As shown above, the impact of invasive species depends on its similarity to native dominants. If the invasive species has similar physiognomy as the native dominants, we can expect it to have rather lower impact on native vegetation and its removal to have similar effect as the removal of native dominant. If the invasive species differs a lot from the native dominant, greater impact and different response to invasive vs. native dominant removal can be expected (Hejda et al., 2009; Hejda and Pyšek, 2006). *I. parviflora* sometimes grows on stands naturally dominated by similar closely related *I. noli-tangere* (Godefroid and Koedam, 2010; Vervoort and Jacquemart, 2012). On such stands, it would be possible and advisable to compare the effect of native and invasive *Impatiens* removal and better quantify the level of damage caused by *I. parviflora*. Similar experiment could be performed with other native dominants growing on the stands with *I. parviflora*. However, *I. parviflora* also quite often grows on stands with no native dominants (Chmura and Sierka, 2006). As this was the case of our study area, performing control experiment removing native dominant was not feasible.

As shown above, the characteristics of the invaded community are very important when assessing the impact of any invasive plant. Moreover, for assessing the impact of an invasive species, we should focus not only on species richness or other measures of species diversity, but also consider specific species composition of the invaded community. Some invasive species might reduce species richness in the area by restricting the growth of other alien plants or widely distributed weeds. An example of such invasives might be *Reynoutria* or *Solidago* species that commonly invade ruderal stands with high proportion of common weeds and alien species (Hejda et al., 2009). On the other side, some invasive species might have overall weaker impact on native communities, but restrict the growth of rare species. An example of such species is *Lupinus polyphyllus*, a species growing among others on oligotrophic species rich mountain meadows (Hejda et al., 2009). In our study, we focused only on one type of community, which does not allow us to make general conclusions. We showed that in oak-hornbeam forests *I. parviflora* affects native vegetation, suppresses the growth of early spring species and reduces species diversity. It, however, did not represent any serious threat to the community simply because there are no rare or protected species in the area. As the species is able to colonize many other types of habitats, some of them of great conservation value, it is desirable to perform similar experiment in other areas as well. The other areas could include either other communities typical for *I. parviflora*, such as floodplain forests or ravine forests (Pyšek et al., 2012b) or less often invaded communities with higher conservation value, such as thermophilous oak forests or steppes on rocks.

As no phytocenological reléves were taken in 2011 and as the vegetation in removal and invaded plots differed already in 2012, we cannot be sure whether the observed differences between removal and invaded plots are caused by *I. parviflora* removal or whether the vegetation differed prior to *I. parviflora* removal. However, the experiment was set up by the end of August, when it was still easy to recognize invaded and non-invaded stand, but when there was nearly no herb layer that could be sampled to get information about initial state of vegetation. If we set up the experiment in spring 2012 after *I. parviflora* germination, we would know the initial species composition, but the experiment would be one season shorter and the plot could be affected by the presence of *I. parviflora* in the beginning of the season. Setting up the experiment in summer 2011 allowed us to remove *I. parviflora* seedlings from the very beginning of the season so any possible effect of *I. parviflora* on native vegetation in removal plots was eliminated. Interpretation of the results would also be easier if the data about surrounding non-invaded vegetation were available (Rejmánek et al., 2013). However, even though the control non-invaded plots were set up in the beginning of the experiment as suggested by Barney et al. (2015), these were colonized by *I. parviflora* in very short time and therefore could not be used for this purpose. Nevertheless, we claim that observed differences between removal and invaded plots are caused by *I. parviflora* removal and we support it by two facts. First, the pairs of invaded plots were placed in a homogenous part of the forest (see Methods for details) and were placed right next to each other, so it can be expected that the initial species composition was more or less the same. Second, the differences between invaded and removal plots that were observed in 2012 became even more pronounced in subsequent years. It is therefore very likely that the changes in vegetation observed in years 2013-2015 started already in 2012, very soon after *I. parviflora* removal.

Conclusions

In the study we showed that *Impatiens parviflora* has negative impact on native vegetation of oak-hornbeam forests. After the invader removal, significant increase in both number and cover of native species was observed. Mostly small early flowering heliophilous species were suppressed by *I. parviflora* invasion, indicating that the impact is due to intensive competition for light in the beginning of the season. However, the vegetation seems to recover quite quickly after the invader removal, indicating that no permanent damage is caused to the ecosystem. As the species can colonize wide range of habitats, it is desirable to perform the same type of study in other types of habitats as well.

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Supplement

Supplement 1: Average cover [%] of individual species in removal and invaded plots in springs and summers 2012-2015.

	spring								summer							
	removal				invaded				removal				invaded			
	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015	2012	2013	2014	2015
Impatiens parviflora	0.00	0.00	0.00	0.00	6.80	30.00	30.99	40.00	0.00	0.00	0.00	0.00	27.96	55.43	55.49	55.49
Anthriscus sylvestris	0.00	10.56	8.33	8.33	0.00	0.00	0.00	0.00	0.00	13.33	13.33	13.33	0.00	0.00	0.00	0.00
Aliaria petiolata	0.00	8.33	8.61	9.17	0.00	0.00	1.67	0.00	0.00	8.69	9.31	11.59	0.00	1.78	1.94	1.94
Acer pseudoplatanus	1.05	0.00	0.00	0.00	0.25	0.00	2.22	0.00	1.60	1.11	2.22	3.33	0.25	0.37	0.37	0.37
Adoxa moschatellina	6.91	11.73	15.56	16.79	9.38	10.00	2.75	10.00	0.00	0.17	0.61	2.36	0.00	0.00	0.00	0.00
Anemone nemorosa	6.67	14.17	18.70	20.65	4.14	0.00	2.41	0.00	0.80	5.74	7.22	8.52	0.00	0.00	0.00	0.00
Carex ovalis	0.00	0.00	12.22	12.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carpinus betulus	1.42	8.58	10.43	10.43	1.42	5.00	3.10	5.00	1.42	3.11	4.20	6.02	1.42	0.26	0.26	0.26
Corydalis cava	7.17	18.83	22.65	23.21	5.49	0.00	1.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Elytrigia repens	0.00	5.56	5.56	5.56	0.00	0.00	0.00	0.00	0.00	3.33	3.33	3.33	0.00	0.00	0.00	0.00
Euonymus europaeus	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ficaria verna	6.42	22.44	26.67	28.00	4.63	0.00	2.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fraxinus excelsior	5.31	8.33	7.50	7.50	5.48	20.00	10.04	20.00	6.11	6.78	8.67	9.22	5.68	7.96	7.59	7.59
Galeobdolon luteum	7.90	12.41	12.04	12.04	1.23	0.00	5.89	0.00	17.04	5.04	5.59	10.00	1.98	3.89	3.89	3.89
Galium aparine	3.52	7.11	7.00	7.00	5.43	10.00	9.89	10.00	0.80	1.89	1.86	2.50	0.00	2.78	3.00	3.00
Geranium robertianum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.12	0.00	0.00	0.00
Geum urbanum	0.43	9.44	8.52	8.52	5.12	0.00	8.33	0.00	3.70	3.33	4.03	5.14	5.00	2.78	3.33	3.33
Maianthemum bifolium	1.73	36.67	40.00	41.11	1.00	0.00	1.50	0.00	1.85	16.11	19.44	21.67	1.23	2.50	0.56	0.56
Poa nemoralis	1.54	6.81	6.81	6.81	2.35	20.00	3.33	20.00	1.98	2.89	3.11	3.78	0.86	1.94	1.94	1.94
Quercus robur	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Stelaria media	0.00	1.67	2.50	2.50	0.00	0.00	0.00	0.00	1.23	0.00	0.00	0.00	0.19	0.00	0.00	0.00
Symphytum tuberosum	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Urtica dioica	0.43	0.00	0.00	0.00	0.00	0.00	1.67	0.00	0.00	0.00	0.00	0.00	0.25	0.00	1.11	1.11
Veronica sublobata	4.07	8.56	7.89	7.89	3.21	0.00	5.28	0.00	0.00	1.37	1.35	3.06	0.28	0.39	0.58	0.58
Viola riviniana	0.99	2.22	2.78	2.78	0.00	0.00	0.00	0.00	0.99	2.22	2.22	3.70	0.00	0.00	0.00	0.00

Supplement 2

See Table S2 and Fig. S2.

Table S2: Dependence of cover of native species on treatment, year and treatment × year interaction. Df Error = 56. Significant results are in bold.

		species cover			
		spring		summer	
	df	p	F	p	F
treatment	1	**0.015**	**7.32**	0.062	3.72
year	3	**<0.001**	**2810.79**	0.154	2.03
treatment×year	3	**<0.001**	**33.43**	**<0.001**	**12.58**

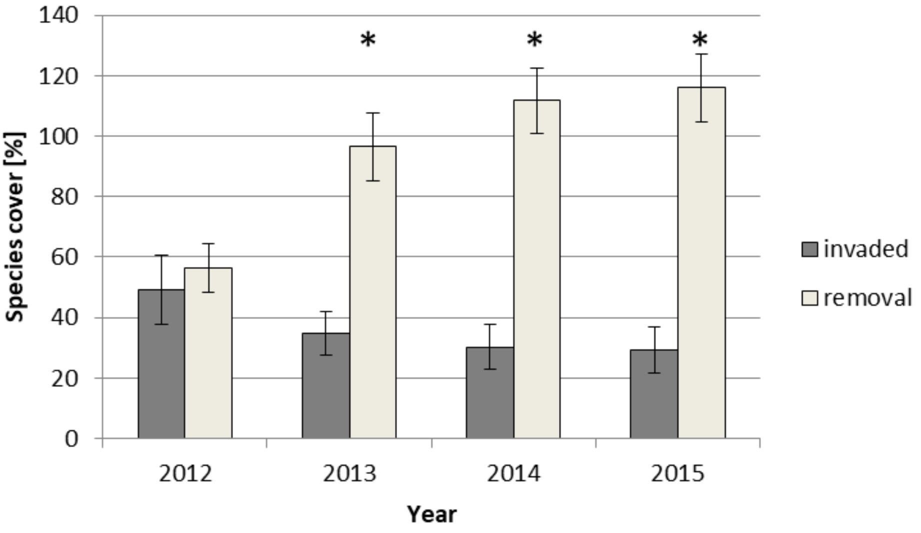

Fig. S2: Cover of native species in invaded and removal plots in spring in each of four sampling years (mean ± SE). Significant differences between removal and invaded plots indicated by an asterisk (*).

Contributions

Contribution of Anna Aldorfová (Florianová) to the papers with co-author included in the thesis:

Study 1: Aldorfová, A., Knobová, P. & Münzbergová, Z. Plant-soil feedback contributes to predicting plant invasiveness of 68 alien plant species differing in invasive status. *Manuscript*.

Idea: **AA**, PK, ZM
Data collection: **AA**, PK
Data analyses: **AA** with help of ZM
Writing: **AA** with help of ZM

Study 2: Aldorfová, A. & Münzbergová. Conditions of plant cultivation affect the differences in intraspecific plant-soil feedback between invasive and native dominants. *Manuscript*.

Idea: **AA**, ZM
Data collection: **AA**
Data analyses: **AA** with help of ZM
Writing: **AA** with help of ZM

Study 3: Florianová, A. & Münzbergová, Z. (2018). The intensity of intraspecific plant-soil feedbacks in alien *Impatiens* species depends on the environment. *Perspectives in Plant Ecology, Evolution and Systematics* 32: 56-64.

Idea: **AF**, ZM
Data collection: **AF**
Data analyses: **AF** with help of ZM
Writing: **AF** with help of ZM

Study 4: Florianová, A. & Münzbergová, Z. (2018). Drivers of natural spread of invasive *Impatiens parviflora* differ between life-cycle stages. *Biological Invasions*, 20: 2121-2140.

Idea: **AF**, ZM
Data collection: **AF**
Data analyses: **AF** with help of ZM
Writing: **AF** with help of ZM

Study 5: Florianová, A. & Münzbergová, Z. (2017). Invasive *Impatiens parviflora* has negative impact on native vegetation in oak-hornbeam forests. *Flora* 226: 10-16.

Idea: **AF**, ZM
Data collection: **AF**
Data analyses: **AF** with help of ZM
Writing: **AF** with help of ZM