Impacts of invasive plants on soil fungi and on above- and belowground plant diversity in temperate forests

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CONTENTS

SUMMARY

Urbanization is considered as a major driver for biotic homogenization and also promotes the dispersal of non-native species. In the last decades, non-native plant species have increasingly invaded Central European forests. The spread of alien plants is generally assumed to be one of the most important threats for native biodiversity and this in turn could affect forest ecosystem functions and services. Understanding how invasive species affect native biodiversity, both above- and belowground, and their impact on ecosystem functioning is of central importance in conservation biology. Within the scope of this thesis, four studies were conducted to examine the introduction pathways of non-native plant species into natural forest habitats and to better understand potential effects of non-native plants on ecosystems by changing plant, soil bacterial and fungal communities.

The aim of the first study was to examine the roles of suburban settlements and of the surrounding landscape composition for the spread of non-native plants into adjacent mixed deciduous forests in Southern and Northwestern Switzerland. For this purpose, I conducted field surveys and recorded the number and abundance of native and non-native species in forest sites situated adjacent to settlements and in control sites far from settlements. In both study regions, non-native species were found in higher number and larger abundance in forests adjacent to settlements than in forests far away from settlements. These findings highlight the role of settlements as an effective source for the spread of non-native plant species into suburban forests. In addition, the occurrence of non-native plants was positively affected by the proximity of roads and by the percentage cover of gardens around the study sites, showing that the composition of the surrounding landscape matrix also affects the establishment of non-native plants.

Apart from understanding the pathways of the introduction of non-native species into natural habitats, assessing and understanding their impact on biodiversity and ecosystem functioning is of crucial importance. Several studies investigated the impacts of non-native, invasive species on aboveground biodiversity, whereas belowground diversity and its role for ecosystem functioning are much less studied in this respect. Therefore, the aim of the second study was to assess the potential impacts of the annual invasive plant species *Impatiens glandulifera* on soil fungal and bacterial communities in forests of Northwestern Switzerland. To do this, I collected soil samples in coniferous and deciduous forest areas invaded by *I. glandulifera* and in forest areas, which were not yet invaded. The diversity and composition of the soil fungal community was assessed using terminal restriction fragment length polymorphism (T-RFLP) analysis. Biolog Ecoplates were used to assess the activity of soil bacterial communities. Forests invaded by *I. glandulifera* were characterized by a higher diversity and an altered composition of the soil fungal community, and by an overall lower

soil bacterial activity in late spring. These can be indirect effects of altered soil properties induced by the presence of the invasive plant, combined with the release of allelopathic compounds into the soil.

Soil fungi fulfil a variety of ecosystem functions. Among them, mycorrhizal fungi develop mutualistic partnerships with the roots of the majority of plant species and play a crucial role for soil nutrient and water uptake by plants, the diversity of the plant communities and forest ecosystem functioning. The third study aimed to investigate the effects of *I. glandulifera* on hyphal growth of ectomycorrhizal fungi, their genetic diversity and the diversity of other soil fungi in deciduous forests. Pairs of transect lines were established at the edge of *I. glandulifera* patches. Using ingrowth mesh bags, I assessed hyphal length along the transects, and applied the T-RFLP analysis to examine fungal genetic diversity. *I. glandulifera* drastically reduced hyphal growth and affected the composition of the soil fungal community, but did not alter their diversity. This may negatively affect nutrient cycling and soil stability.

Disregarding the fact that the majority of ecosystems have more than 50% of plant biomass belowground, most studies investigating the effects of invasive species on plant diversity focused only on the aboveground vegetation. The aim of the fourth study was to assess the potential impact of invasive plants on belowground plant species richness in deciduous forests. This has not yet been examined in any study. I established plots in forests invaded by *I. glandulifera* and in forests, which were not yet invaded. In each plot, I conducted vegetation surveys to assess aboveground plant diversity. Belowground species richness was determined by collecting root samples and by applying the fluorescent amplified fragment length polymorphism (FAFLP) technique for two regions of the chloroplast DNA. Plant species richness was reduced above- but not belowground in invaded forests, whereas plant species composition differed between invaded and uninvaded forests both above- and belowground. Root biomass was strongly reduced in forests invaded by *I. glandulifera*, and this may negatively affect their soil stability and productivity. These results show that the impact of invasive plants may differ between above- and belowground plant communities.

The findings of this thesis highlight the role of settlements for the spread of non-native plant species into adjacent forests. By focusing on belowground diversity, my studies show that annual invasive plants have the potential to disturb belowground fungal, bacterial and plant communities. As a consequence, ecosystem services and functions of invaded forest habitats, such as nutrient cycling, productivity and soil stability, may also be influenced. Removal of existing *I. glandulifera* populations and preventing further spread of this invasive plant may be a worthy investment for the conservation of native species diversity and the functioning of forest ecosystems.

GENERAL INTRODUCTION

The intentional and unintentional introduction of non-native species is considered as a major threat to native biodiversity (Pimentel et al., 2005; Pejchar and Mooney, 2009). Non-native species have the potential to affect ecosystems by changing species diversity, community structure and interactions between organisms, sometimes causing local extinction of native species (Vilà et al., 2011; Pyšek et al., 2012). During the last decade, non-native plants have increasingly invaded deciduous and coniferous forests in Central Europe (Wagner et al., 2017). Forests harbour 27% of all naturalized non-native species, and more than 50% of the non-native plant species occurring in the wild were mainly imported for ornamental or horticultural purposes (Lambdon et al., 2008). A number of studies documented the spread and expansion of non-native species into different habitats. Urbanization can promote the dispersal of non-native plant species, which may result in biotic homogenization (McKinney, 2002; Kühn and Klotz, 2006). Several studies investigated the mechanisms driving plant invasions into natural habitats in urban environments, but the influences of settlements and of the surrounding landscape matrix on the spread of non-native species into forests were rarely investigated in Europe (for exceptions see: Gonzàlez-Moreno et al., 2013a,b). Apart from understanding how non-native species come into natural habitats, it is also important to assess and understand their impact on biodiversity and ecosystem functioning. There is increasing evidence that ecosystem functioning is positively affected by the "aboveground" biodiversity, but the relationships between "belowground" diversity and ecosystem functioning and services are much less studied. Furthermore, it is not known to which extent invasive plants can disturb "belowground" diversity and ecosystem functions in forests.

In my dissertation I investigated various aspects of plant invasion ecology. **Chapter I** focuses on non-native plant species in general, and on how their distribution in forests is influenced by the surrounding settlements, whereas **Chapters II**, **III**, and **IV** concentrate on one particular invasive non-native plant species, namely *Impatiens glandulifera* Royle (Himalayan balsam), and on how this species affects plant diversity and soil fungal and bacterial communities in forests. *I. glandulifera* is an herbaceous annual plant belonging to the family Balsaminaceae, which is native in the western Himalaya and was introduced as garden ornamental plant to Europe and North America in the middle of the 19th century (Beerling and Perrins, 1993). It became naturalized and invasive in riparian and disturbed habitats (Hejda and Pyšek, 2006). In the last decades, *I. glandulifera* has increasingly invaded deciduous and coniferous forests, owing to natural and man-related disturbances (Wagner et al., 2017). The species has been classified as an invasive alien species of Union concern by the European commission in 2017 (European Union, 2017). *I. glandulifera* is able to alter physical and chemical soil characteristics in forests (Ruckli et al., 2013, 2014a; Rusterholz et

al., 2014) and to affect the composition of soil invertebrate (Tanner et al., 2013; Rusterholz et al., 2014) and gastropod communities (Ruckli et al. 2013). It can also disturb soil fungal communities and negatively affect mycorrhizal symbioses of tree saplings, resulting in a higher sapling mortality and in a reduced forest regeneration (Tanner & Gange, 2013; Ruckli et al. 2014a, 2016; Pattison et al. 2016). Ruckli et al. (2014b) identified the allelopathic compound 2-methoxy-1,4-naphthoquinone in roots and leaves of *I. glandulifera*, which is released into the soil and has strong inhibitory effects on the growth of mycorrhizal fungi and the germination of several native herbs. This indicates that naphthoquinone release may contribute to the invasion success of *I. glandulifera* and thus supports the "novel weapons hypothesis" (Callaway and Ridenour, 2004).

Several studies investigated the effects of *I. glandulifera* on aboveground plant species richness and composition, both in forests and in river banks. Even if *I. glandulifera* seems to cause slight changes in plant species richness and shifts in plant species composition both in forest and riparian habitats (Maule et al., 2000; Hejda and Pyšek, 2006; Hulme and Bremner, 2006; Diekmann et al., 2016), results are contradictory. According to Rusterholz et al. (2017), *I. glandulifera* negatively affects both the aboveground vegetation and the soil seed bank in forests with a delay of about 15 years after the invasion.

I investigated the effects of *I. glandulifera* on plant diversity (both above- and belowground) and on soil fungal and bacterial communities in a forest 15 km south of Basel, Northwestern Switzerland. The forest was affected by the windstorm Lothar in 1999, and *I. glandulifera* started to invade several sites shortly after the storm in spring 2000.

Focus of the thesis

The main aim of this thesis is (1) to examine the role of settlements for the distribution of non-native plant species in forests, and (2) to assess how one of these non-native species, *I. glandulifera*, can influence several aspects of the belowground diversity in forest ecosystems, and thus affect ecosystem functioning and services. To address the first topic, I conducted vegetation surveys in mixed deciduous forest sites adjacent to settlements, and in sites far away from settlements, in proximity of a meadow and with no buildings in the surroundings. This was done in two distinct regions of Switzerland, one in its Southern part (region of Lugano) and one in the Northwestern part (region of Basel). I expected differences in the extent of the occurrence of non-native species between the two regions, because Southern Switzerland has a longer history of introductions of non-native plant species (Schröter, 1936), is exposed to a higher propagule pressure both from gardens, which harbour more non-native species, as well as from the Mediterranean area, and has a milder winter climate than Northwestern Switzerland. To evaluate the influence of single landscape

components on the frequency and abundance of non-native plant species in suburban forests, various habitat and landscape characteristics were assessed in the surroundings of the study sites (100 m radius). The results of this study are presented in **Chapter I**.

A main focus of my thesis is on belowground communities in forest ecosystems, and on how these are influenced by the invasion of non-native plants. Soil fungi, for example, are a key component of belowground communities and are involved in a variety of microbiological and ecological processes, influencing soil fertility, decomposition, cycling of minerals and organic matter (Itoo and Reshi, 2013). They can be classified to be mutualistic, saprophytic, endophytic or pathogenic (Dighton, 2016). The main part of soil fungi, in terms of biomass, is represented by mycorrhizal fungi (Nehls, 2008), which constitute a mutualistic symbiosis between soil fungi and plants. Mycorrhizal fungi play a crucial role for the establishment, survival and growth of vascular plants including trees and for the regeneration of forests. Together with soil fungi, soil bacteria play a key role in energy flow, nutrient cycling and organic matter turnover (Bauhus and Khanna, 1999). There is increasing evidence that invasive plants can affect soil fungal and bacterial communities, but the majority of studies in this sense were carried out in grassland ecosystems (e.g. Hawkes et al., 2006; Mummey and Rillig, 2006; Zubek et al., 2016). Some studies were conducted in Asian (e.g. Niu et al., 2007) and North-American forests (e.g. Stinson et al., 2006; Wolfe et al., 2008; Barto et al., 2011), whereas Central European forests are underrepresented in this respect. **Chapter II** presents the results of a field survey, which investigated the effects of the invasive plant *I. glandulifera* on species richness and composition of soil fungal communities and on the activity and composition of the soil bacterial community in deciduous forests dominated by *Fagus sylvatica*, and in coniferous forests dominated by *Picea abies* or *Abies alba*, the most abundant forest types in Switzerland and Central Europe.

Mycorrhizal fungi can be involved in so called 'mycorrhizal networks', defined as fungal hyphae that connect the roots of at least two plants, and that are able to redistribute limited resources among individuals of different plant species (Newman, 1988; Simard et al., 2012; Horton, 2015). Invasive species like *Alliaria petiolata* have the potential to affect the growth of mycorrhizal mycelia and the formation of mycorrhizal networks (Wolfe et al., 2008), but studies in this respect are scarce. **Chapter III** presents the results of a study, in which I assessed the effects of *I. glandulifera* on the growth of ectomycorrhizal hyphae in the field, using the 'ingrowth mesh bag' method (Wallander et al., 2001), and applied genetic analyses to examine the diversity and composition of ectomycorrhizal fungi and other soil fungi along 3-m long transect lines placed perpendicular to the edge of *I. glandulifera* patches in mixed deciduous forests.

An other important component of belowground ecosystems is represented by plant structures: even if most studies regarding plant communities and their role for ecosystem

functioning are based on data of aboveground vegetation, the majority of ecosystems have more than 50% of plant production or biomass belowground (Jackson et al., 1997; Poorter et al., 2012). Plant root diversity, for example, plays a key role for soil stability and productivity. Also studies investigating the effects of invasive plant species on native plant communities classically concentrate on the aboveground vegetation (Richardson et al., 1989; Hejda et al., 2009). Problems of such aboveground approaches include, for example, the overlooking of spring geophytes that already completed their cycle, or the presence of roots from neighbour plants that do not occur aboveground in the survey plot, but belowground contribute to soil structure and stability. To my knowledge, no study investigated so far the potential impact of invasive plants on the belowground plant species richness. In the past, any determination of belowground plant species richness has been hindered by methodological difficulties. Nowadays, however, a number of DNA-based methods are available, which allow the determination of belowground plant species richness in the field (e.g. 'fluorescent amplified fragment length polymorphism' technique, FAFLP; Taggart et al., 2011). By applying these genetic techniques, I assessed the potential effects of *I. glandulifera* on the belowground plant diversity, by comparing both above- and belowground plant diversity in invaded and uninvaded mixed deciduous forests. The results of this study are presented in **Chapter IV**.

In the final section of this thesis, the **General Discussion**, I discuss the most important findings of the four chapters and their implications for sciences as well as for the management of the invaded areas.

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Chapter I

Settlements as a source for the spread of non-native plants into Central European suburban forests

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Settlements as a source for the spread of non-native plants into Central European suburban forests

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ABSTRACT

Urbanization is considered as a major driver for biotic homogenization. Urbanization also promotes the dispersal of non-native species. This study examined the roles of suburban settlements and of the surrounding landscape composition for the spread of non-native plant species into adjacent mixed deciduous forests in Southern and Northwestern Switzerland. The number and abundance of native and non-native vascular plant species in both the ground vegetation and shrub layer were recorded in 15 forest sites situated adjacent to settlements and 15 control sites far from settlements. Various site and landscape characteristics were assessed in the surroundings (100 m radius) of the study sites. In both regions we found a higher number and larger abundance of non-native plant species in forest sites adjacent to settlements than in control forest sites. Furthermore, non-native plants were more frequently recorded close to roads and in sites surrounded by a large percentage cover of garden. All these effects were more pronounced in Southern Switzerland, a region with milder winter climate, than in Northwestern Switzerland. Our study showed that settlements are a source for the spread of non-native plant species into Central European suburban forests, and that the composition of the surrounding landscape matrix (e.g. traffic infrastructure, percentage cover of gardens) also affects the establishment of non-native plants.

1. Introduction

The intentional and unintentional introduction of non-native species is considered as a major threat to native biodiversity (Pimentel et al., 2005; Pejchar and Mooney, 2009). Non-native species have the potential to affect ecosystems by changing species diversity, community structure and interactions between organisms, sometimes causing local extinction of native species (Vilà et al., 2011; Pyšek et al., 2012; Stoll et al., 2012). In Europe, more than 50% of the non-native plant species occurring in the wild were mainly imported for ornamental or horticultural purposes (Lambdon et al., 2008; Kowarik, 2010).

Urbanization is also affecting native plant diversity (Knapp et al., 2010) by being a major driver for biotic homogenization (Kühn and Klotz, 2006) and promoting the dispersal of non-native plant species (McKinney, 2002). The spread of non-native plants into adjacent natural habitats (e.g. forests) is influenced by the proximity (Duguay et al., 2007; Gavier-Pizarro et al., 2010) and the size of settlements (Sullivan et al., 2005). In general, the frequency of non-native plants in natural habitats is increased in human-dominated landscapes (Vilà and Ibáñez, 2011; Heinrichs and Pauchard, 2015).

Several studies showed that certain landscape characteristics facilitate the invasion of non-native plant species into natural habitats (González-Moreno et al., 2013a; Vakhlamova et al., 2014). For example, gardens harbour a high variety of ornamental non-native plants, and are therefore important sources for the dispersal of propagules into natural habitats such as forests (Sullivan et al., 2005; Smith et al., 2006). Also traffic infrastructure plays an important role in the dispersal of non-native plants, by increasing disturbance and providing effective dispersal corridors (Von der Lippe et al., 2005; Vakhlamova et al., 2016). Furthermore, human-caused global warming contributes to the invasion success of non-native species (Walther et al., 2001). Numerous ornamental and horticultural non-native plants were introduced from warmer climate regions into Central Europe (Kowarik, 2010). These plants may further benefit from a reduced number of frost days and lower frost intensities predicted in climate change scenarios and can therefore expand into regions, in which they could not survive and

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reproduce under the present climatic conditions (Von der Lippe et al., 2005).

The influences of settlements and of the surrounding landscape matrix on the spread of non-native species into forests were scarcely investigated in Europe (for exceptions see: González-Moreno et al., 2013a,b). In Switzerland, a strong suburbanization trend started in the 1950ies (Kahsai and Schaeffer, 2010). At present, forests represent the most abundant semi-natural habitat in suburban areas, but also harbour 27% of all naturalized non-native species (Lambdon et al., 2008). The aim of the present study was to assess the influence of the proximity of settlements on the frequency and abundance of non-native plant species in forests situated in two distinct regions of Switzerland, one in its Southern part and one in the Northwestern part. Southern Switzerland (Canton Ticino) has a longer history of introductions of non-native plant species (Schröter, 1936), is exposed to a higher propagule pressure both from gardens, which harbour more non-native species, as well as from the Mediterranean area, and has a milder winter climate than Northwestern Switzerland (region of Basel). We therefore expect a higher frequency of non-native plants in forests near settlements in Southern Switzerland than in Northwestern Switzerland. We tested the following hypotheses:

1) The number and percentage of non-native plant species in the ground vegetation and shrub layer are increased in forests in proximity to settlements compared to forests further away from settlements.
2) Landscape features in the surroundings of forests near settlements (e.g. percentage cover of private gardens, distance to the nearest road) also influence the number and percentage of non-native plant species in suburban forests.
3) The number and abundance of non-native plant species in forests near settlements decreases from the edge to the forest interior.

2. Materials and methods

2.1. Study sites

The study was conducted in mixed deciduous forests at 15 localities situated in two suburban regions of Switzerland (Fig. 1; Table S1 – Online Supplementary Material). Seven localities were situated in the surroundings of Lugano (46°00′ N, 8°57′ E) in Southern Switzerland, eight localities near Basel (47°32' N, 7°34' E) in Northwestern Switzerland (Fig. 1). The region of Lugano has a mean annual temperature of 12.4 °C and a mean annual precipitation of 1559 mm (Meteo Swiss, 2013). The region of Basel has a mean annual temperature and precipitation of 10.5 °C and of 842 mm, respectively (Meteo Swiss, 2013). The two regions are situated 200 km apart, separated by the Alps. In both regions, the localities were situated within an area of 5 km × 15 km, with a minimum distance of 800 m between two localities. Elevation ranged from 319 to 536 m a.s.l. in the region of Lugano, and from 313 to 515 m a.s.l. in the region of Basel.

2.2. Design of the survey

To examine the impact of the proximity of settlement on the occurrence and abundance of non-native plant species in forests, we selected a site adjacent to settlements in each locality (hereafter 'near-settlement site') and another site 200–960 m (mean 500 m) apart, in proximity of a meadow and with no buildings in the surroundings (hereafter 'control site' or 'far-from-settlement site'; Fig. S1). Within region, near-settlement sites and control sites had similar soil characteristics (Table S2), elevation, inclination, forest types and forest management.

In each study site, an area of 26 m × 22 m, which was subdivided into three zones, was setup (Fig. 2). Zone 1 was a 6-m wide strip

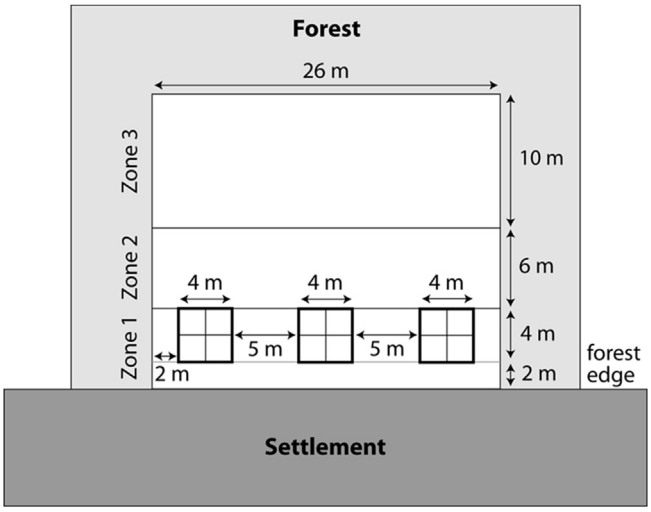

Fig. 2. Layout of the survey design to investigate the effect of proximity of settlement on the spread of non-native plant species into forests. Control sites were located in forests adjacent to meadows and with no buildings in the close surroundings.

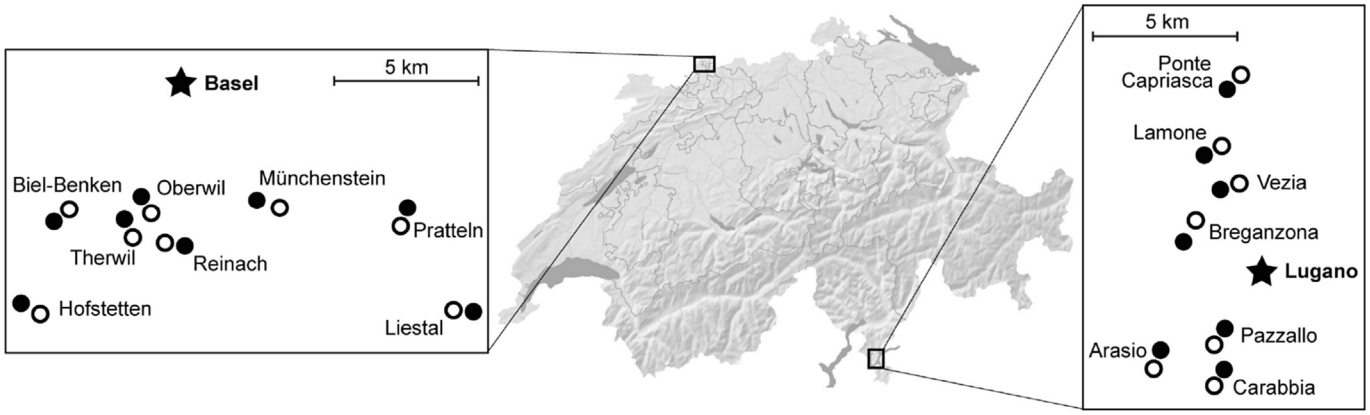

Fig. 1. Location of the near-settlement (filled circles) and control (far-from-settlement) sites (open circles) in the regions of Lugano (right) and Basel (left), Switzerland.

adjacent to the forest edge. This zone was followed by the 6 m-wide zone 2 and the 10-m wide zone 3 (Fig. 2). Three sampling plots (4 m × 4 m) were installed in a row in zone 1, with a distance of 2 m to the forest edge to avoid potential edge effects. The distance between the plots was 5 m (Fig. 2).

2.3. Plant survey

The species composition of the forest vegetation was examined on two different spatial scales. At the plot level, richness and abundance of single plant species belonging to the above-ground vegetation were assessed. Each of the three 4 m × 4 m plots installed in zone 1 in each study site was subdivided in four subplots (2 m × 2 m). In each plot, one of the subplots with a distance of 2 m to the forest edge was randomly chosen. In this subplot, all plants in the ground vegetation (herbs and woody plants up to a height of 40 cm) were determined to the species and their cover was estimated using the Braun-Blanquet (1964) scale. To complete the species list of this sampling plot, the other three subplots were carefully searched for additional species. Thus, the abundance of plant species was based on one subplot of 2 m × 2 m, while for the records of plant species richness the entire plot (4 m × 4 m) was considered. Furthermore, the plants belonging to the shrub layer (woody plants, with a height between 40 cm and 2.5 m) were determined to the species and the number of individuals for each species was counted in each plot (4 m × 4 m). Finally, the total cover of the ground vegetation, of leaf litter, dead wood and bare ground were visually estimated (accuracy 5%) and the girth (cm) of trees present in the plot was measured at breast height in each plot.

Since most non-native species were patchily distributed, at the study site level all three zones were searched for non-native species, both in the ground layer, and in the shrub layer, and their numbers were counted. Their abundances were assigned to one of the following classes: I: 1–5 individuals, II: 6–10, III: 11–50, IV: 51–100, V: 101–200 and VI: >200 individuals. The zones allowed an assessment of potential effects of the distance to the forest edge. Species composition, the abundance and the girth of trees were also assessed in the three zones.

Plant species were identified and classified as native or non-native to Switzerland according to Fitschen (2007) and Lauber et al. (2012). Plant surveys were carried out between April and September 2014, once in spring and once in autumn.

2.4. Soil characteristics

To assess the soil characteristics of the study sites, five soil samples were taken in each zone of a site using a soil corer (depth 5 cm; diameter 5 cm; volume 100 cm^3) in August–September 2014. The soil samples were taken approximately 4 m apart in the central part of each zone. Soil samples obtained from each zone were pooled and mixed, resulting in a total of 90 samples (three zones x 15 localities x two treatments [near-settlement and control sites]). The soil samples were sieved (mesh size 2 mm) and dried for 48 h at 50 °C. Soil moisture (%) was determined using the fresh weight to dry weight ratio. Soil pH was assessed in distilled water (1:2.5 soil:water) (Allen, 1989). Total soil organic matter content (%) was determined as loss-on-ignition of oven-dried soil at 750 °C for 16 h (Allen, 1989).

2.5. Site and landscape structure characteristics

For each near-settlement and control site, one site and six landscape characteristics were assessed (Table S3). Aerial imageries were used to measure the percentage area covered by forests, agriculture land, traffic infrastructure, built-up area and ornamental gardens in the present-day situation within radii of 100 m and 200 m around the central plot of each near-settlement and control site (http://www.map.geo.admin.ch/; date: 21 October 2014; scale 1:2′500). The percentage area covered by the different landscape elements was determined using the pixel counting function of Adobe Photoshop, version 10.0.1 (estimated to the nearest 1%). In addition, the length (m) of the forest-urban interface was measured within radii of 100 m and 200 m (interface between forest and buildings or ornamental gardens) and the distance (m) from the study site to the nearest road was also assessed.

2.6. Data analyses

Statistical analyses were performed in R, version 3.1.2 (R Core Team, 2014). Our study sites in the regions of Lugano and Basel only shared 29% of the plant species recorded. Therefore the data were analysed separately for both regions.

Linear mixed-effect models (LME) were used to analyse the effects of proximity to settlements (near to or far from settlements) and plot characteristics on the total number of plant species, and on both the number and percentage of non-native species. To avoid pseudoreplication, proximity to settlement was nested in location and included as fixed factor, whereas plot was nested in site as random factor. Plot characteristics were included in the models as cofactors. Three plot characteristics were excluded from the models because of intercorrelations (Table S4). Soil characteristics were not included in the models because they did not differ between near-settlement and control sites in both regions (Table S2).

Because site and landscape variables differed between near-settlement and control sites (Principal Components Analysis; data not shown), separated LME models were used to analyse their influence on total plant species richness, and on the number and percentage of non-native species at the plot level. Plot was nested in site, site nested in location, and both included as random factors, while site and landscape characteristics were included as cofactors. Three landscape characteristics were excluded from the models because of intercorrelations (Table S5). At the site level, similar LME analyses were conducted to investigate the effects of site and landscape characteristics on the number and abundance of non-native plant species in the three zones. For the number of non-native plant individuals, median values of the abundance classes were used. Site was nested in location and included as random factor, whereas both site and landscape characteristics were included as cofactors. Since LME analyses conducted at site level (Table S6) yielded similar results as the analyses at plot level, only the results at the plot level are presented.

To assess whether the distance to the forest edge affects the number and abundance of non-native plant species in the three zones of a study site, generalized linear models (GLM) with quasi-Poisson distributed errors were applied. Proximity to settlements (nested in location) and zone were included as fixed factors, whereas basal tree area was included as cofactor. All models were stepwise reduced according to Crawley (2007).

All statistical analyses were conducted twice: for the ground vegetation and the shrub layer. Since LMEs and GLMs conducted on the landscape scales of 100 m and 200 m radii yielded similar results, only the results of the analyses on the scale of 100 m are presented.

To assess whether proximity to settlements affects plant species composition at the subplot level, non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity measure was applied. The ordinations were fitted using the *isoMDS* function with default options on two dimensions in the *vegan* package in R. In a second step, proximity to settlements was fitted onto the

ordinations of plants using the function *envfit* with 999 permutations in the *vegan* package in R (Oksanen et al., 2013). NMDS analysis was conducted at two different levels: at the subplot level (4 m^2) and at the site level (three subplots pooled, 12 m^2, with mean abundance data).

3. Results

3.1. Species richness

In the region of Lugano, out of a total of 102 plant species recorded in the ground vegetation, 79 species (77.5%) were found in near-settlement sites (S) and 69 (67.6%) in control sites (C) (Table S7). Altogether, 25 species (24.5%) were non-native to Switzerland (S: 22, C: 12; Table S8). In the shrub layer, 44 species were found in the sites near Lugano (S: 30, C: 29), 14 of them (31.8%) were non-native (S: 12, C: 4; Table S8).

A total of 72 plant species were recorded in the ground vegetation in the sites near Basel, 63 (87.5%) in near-settlement sites and 52 (72.2%) in control sites (Table S7). Twelve of them (16.7%) were non-native to Switzerland (S: 10, C: 6; Table S8). In the shrub layer, 30 species were found (S: 24, C: 24), 5 of them (16.7%) were non-native (S: 4, C: 1; Table S8).

3.2. Effect of proximity to settlements on species richness

In the region of Lugano, total number of plant species, as well as the number and percentage of non-native species in both ground vegetation and shrub layer were higher in near-settlement sites than in control sites (Figs. 3 and 4; Table 1).

In the region of Basel, the total number of plant species in the ground vegetation was not affected by the proximity to settlements (Fig. 3; Table 1a). In contrast, the number and percentage of non-native species in the ground vegetation were higher in near-settlement sites than in the corresponding control sites (Fig. 3; Table 1a). In the shrub layer, species richness was affected by the proximity to settlements (Fig. 4, Table 1b). Species richness in the shrub layer also increased with increasing cover of ground vegetation (Spearman rank correlation: $r_S = 0.44$, $n = 16$, $p = 0.002$) and was influenced by basal tree area (Table 1b), a proxy for light availability.

3.3. Effect of site and landscape characteristics on species richness

In the region of Lugano, total plant species richness in the ground vegetation was influenced by the percentage cover of traffic infrastructure in the close surroundings (Table 2a). Furthermore,

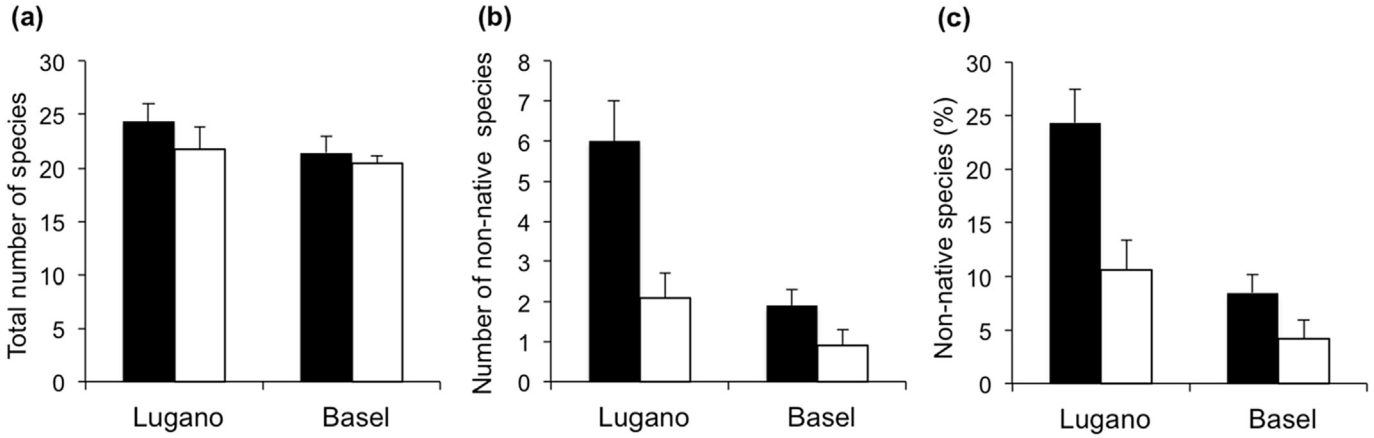

Fig. 3. Total number of plant species (**a**), number of non-native species (**b**), and percentage of non-native species (**c**) recorded in the ground vegetation of near-settlement sites (black bars) and control sites (open bars) in the regions of Lugano (each $n = 7$) and Basel (each $n = 8$), Switzerland. Means ± SE are shown.

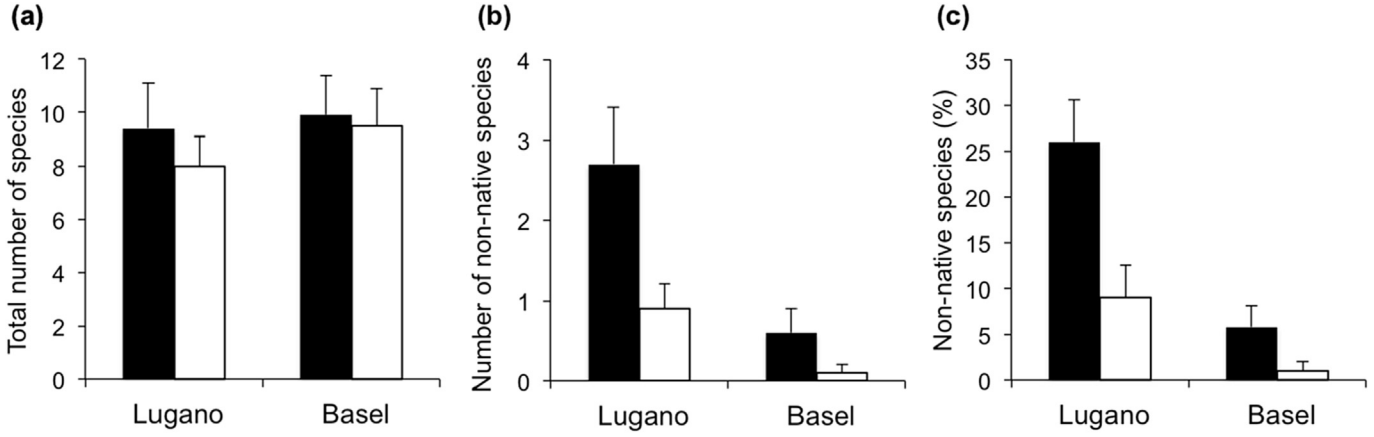

Fig. 4. Total number of plant species (**a**), number of non-native species (**b**), and percentage of non-native species (**c**) recorded in the shrub layer of near-settlement sites (black bars) and control sites (open bars) in the regions of Lugano (each $n = 7$) and Basel (each $n = 8$), Switzerland. Means ± SE are shown.

Table 1
Summary of linear mixed-effect model (LME) analyses showing the effect of proximity to settlement on total number of plant species, number of non-native plant species and percentage of non-native plant species recorded in the ground vegetation (**a**), and in the shrub layer (**b**) in localities near Lugano (near-settlement sites: 7/control sites: 7) and Basel (8/8), Switzerland. Data were analysed at the plot level for each region separately.

	Lugano									Basel								
	Total number of plant species			Number of non-native plant species			Percentage of non-native plant species			Total number of plant species			Number of non-native plant species			Percentage of non-native plant species		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
(a) Ground vegetation																		
Locality	3.41	6, 21	**0.017**	4.42	6, 23	**0.004**	4.61	6, 23	**0.003**	0.94	7, 27	0.491	3.37	7, 27	**0.010**	1.21	7, 27	0.332
[Proximity to settlement]Locality	3.32	7, 21	**0.015**	9.87	7, 23	**<0.001**	11.02	7, 23	**<0.001**	1.91	8, 27	0.099	5.93	8, 27	**<0.001**	2.95	8, 27	**0.017**
Vegetation cover (%)	1.65	1, 21	0.212	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Basal area trees (m^2)	2.48	1, 21	0.130	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(b) Shrub layer																		
Locality	6.08	6, 23	**0.001**	3.92	6, 22	**0.008**	2.95	6, 22	**0.029**	13.16	7, 25	**<0.001**	0.91	7, 26	0.513	0.77	7, 26	0.615
[Proximity to settlement]Locality	2.32	7, 23	0.060	3.51	7, 22	**0.011**	3.41	7, 22	**0.013**	3.32	8, 25	**0.010**	1.42	8, 26	0.237	1.12	8, 26	0.385
Vegetation cover (%)	–	–	–	1.98	1, 22	0.174	3.62	1, 22	0.070	11.02	1, 25	**0.003**	2.76	1, 26	0.108	4.12	1, 26	0.053
Basal area trees (m^2)	–	–	–	–	–	–	–	–	–	6.95	1, 25	**0.014**	–	–	–	–	–	–

– Excluded from the model after step-wise reduction.
Significant *P*-values (<0.05) are indicated in bold.

Table 2
Summary of linear mixed-effect model (LME) analyses showing the effects of site and landscape characteristics (cover in % of different landscape elements within a radius of 100 m around each study site) on total number of plant species, number of non-native plant species and percentage of non-native plant species recorded in the ground vegetation (**a**), and in the shrub layer (**b**) in localities near Lugano (near-settlement sites: 7/control sites: 7) and Basel (8/8), Switzerland. Data were analysed at the plot level for each region separately.

	Lugano									Basel								
	Total number of plant species			Number of non-native plant species			Percentage of non-native plant species			Total number of plant species			Number of non-native plant species			Percentage of non-native plant species		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
(a) Ground vegetation																		
Distance to nearest road (m)	–	–	–	16.29	1, 33	**<0.001**	22.31	1, 33	**<0.001**	1.41	1, 41	0.242	–	–	–	–	–	–
Forest cover (%)	–	–	–	2.10	1, 33	0.157	2.04	1, 33	0.163	–	–	–	5.93	1, 39	**0.020**	4.32	1, 39	**0.044**
Cover of traffic infrastructure (%)	4.39	1, 35	**0.043**	–	–	–	–	–	–	–	–	–	7.65	1, 39	**0.009**	6.59	1, 39	**0.014**
Cover of garden (%)	–	–	–	10.61	1, 33	**0.003**	7.72	1, 33	**0.009**	–	–	–	2.62	1, 39	0.114	1.04	1, 39	0.314
(b) Shrub layer																		
Distance to nearest road (m)	1.64	1, 34	0.210	1.91	1, 34	0.175	1.79	1, 33	0.190	–	–	–	–	–	–	1.05	1, 40	0.311
Forest cover (%)	–	–	–	–	–	–	1.39	1, 33	0.248	12.22	1, 41	**0.001**	1.07	1, 40	0.306	–	–	–
Cover of traffic infrastructure (%)	–	–	–	–	–	–	–	–	–	–	–	–	3.52	1, 40	0.068	2.16	1, 40	0.149
Cover of garden (%)	2.14	1.34	0.152	10.98	1, 34	**0.002**	6.99	1, 33	**0.012**	–	–	–	–	–	–	–	–	–

– Excluded from the model after step-wise reduction.
Significant *P*-values (<0.05) are indicated in bold.

both the number and percentage of non-native species in the ground vegetation were high close to roads and also in study sites surrounded by a large percentage cover of garden (Table 2a). In the shrub layer, both the number and percentage of non-native species were high when a large percentage cover of garden occurred in the surroundings (Table 2b).

In the region of Basel, the number and percentage of non-native species in the ground vegetation were influenced by the percentage cover of forest (Table 2a). Furthermore, both the number and percentage of non-native species were high in sites surrounded by a large percentage cover of traffic infrastructure. In contrast, the total number of plant species was not affected by any of the assessed site and landscape characteristics (Table 2a). In the shrub layer, the total number of species was high when a large percentage cover of forest occurred in the surroundings (Table 2b).

3.4. Influence of the distance to the forest edge

In the region of Lugano, both the number of non-native plant individuals and the number of non-native species in the ground vegetation increased in proximity to settlements and to the forest edges (Table 3a, Table S9), and were affected by the basal tree area. Similar results were found in the shrub layer (Table 3b, Table S9).

Analyses of data from the sites in the region of Basel revealed similar results (Table 3, Table S9).

3.5. Community structure

Despite the difference between near-settlement and control sites in the total plant species richness and non-native plant species richness found in LME analyses, NMDS analyses showed that the species composition of the ground vegetation did not differ between pairs of sites, both at the subplot level (4 m^2), and at the site level (12 m^2), neither in the region of Lugano nor in Basel (Fig. S2).

4. Discussion

Our study showed that sites in deciduous forests near settlements harboured a higher species richness and larger abundance of non-native plants than forest sites far from settlements. The

Table 3
Summary of generalized linear model (GLM) analyses showing the effects of proximity to settlement and distance to the forest edge on the number of non-native plant individuals and number of non-native species recorded in the ground vegetation (**a**), and in the shrub layer (**b**) in localities near Lugano (near-settlement sites: 7/control sites: 7) and Basel (8/8), Switzerland. Data were analysed at the level of zones (three zones within site) for each region separately.

	Lugano						Basel					
	Number of non-native plant individuals			Number of non-native plant species			Number of non-native plant individuals			Number of non-native plant species		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
(a) Ground vegetation												
Locality	23.63	6, 35	**<0.001**	4.87	6, 35	**0.002**	15.81	7, 40	**<0.001**	3.51	7, 40	**0.008**
[Proximity to settlement]Locality	20.19	7, 25	**<0.001**	8.09	7, 25	**<0.001**	15.76	8, 29	**<0.001**	11.74	8, 29	**<0.001**
Distance to the forest edge (zone)	22.13	2, 33	**<0.001**	22.97	2, 33	**0.003**	6.67	2, 38	**0.004**	45.56	2, 38	**<0.001**
Basal area trees (m^2)	27.64	1, 32	**<0.001**	10.98	1, 32	**<0.001**	16.70	1, 37	**<0.001**	10.38	1, 37	**0.003**
(b) Shrub layer												
Locality	5.45	6, 35	**0.001**	4.45	6, 35	**0.003**	12.05	7, 40	**<0.001**	15.16	7, 40	**<0.001**
[Proximity to settlement]Locality	11.28	7, 25	**<0.001**	4.04	7, 25	**0.004**	12.61	8, 30	**<0.001**	10.46	8, 29	**<0.001**
Distance to the forest edge (zone)	8.05	2, 33	**0.002**	17.21	2, 33	**<0.001**	27.18	2, 38	**<0.001**	33.52	2, 38	**<0.001**
Basal area trees (m^2)	14.27	1, 32	**<0.001**	5.03	1, 32	**0.034**	–	–	–	3.53	1, 37	0.070

– Excluded from the model after step-wise reduction.
Significant *P*-values (<0.05) are indicated in bold.

abundance of non-native plants was also influenced by the distance to the nearest road and percentage cover of garden in the close surroundings. Furthermore, both the number and abundance of non-native plant species decreased from the forest edge to the interior. Moreover, the effect of settlements on the frequency of non-native plants was more pronounced in Southern than in Northwestern Switzerland.

4.1. Effect of proximity to settlements on species richness

Our finding that species richness and abundance of non-native plants in forests are affected by the proximity to settlements is supported by other studies conducted in New Zealand (Sullivan et al., 2005), North America (Kuhman et al., 2010), and Spain (González-Moreno et al., 2013b). At a macroecological scale (520 1-km^2 plots regularly distributed over the area of the country), Nobis (2008) reported that invasive plant species richness in Swiss forests increased with increasing percentage cover of settlements in the surroundings. The increase of non-native plant species in forests near settlements can be explained by the fact that domestic gardens are sources of propagules of non-native plants (Sullivan et al., 2005; Marco et al., 2008). Furthermore, the high frequency of disturbances from roads, nearby situated settlements and from recreational activities facilitate the establishment of non-native plants in forests (Gavier-Pizarro et al., 2010; McWilliam et al., 2010a).

However, we did not find any differences in plant species composition between near-settlement and control sites. This unexpected result can be explained by the low frequencies of non-native plant individuals found in the study sites.

Our study also showed that the number and abundance of non-native plant species decreased from the edge to the forest interior at sites in proximity to settlements, supporting earlier findings of Honnay et al. (2002) and Vilà and Ibáñez (2011). In fact, the impacts of residential encroachment (e.g. garden waste disposal, recreational activities, residents access) are primarily concentrated along forest edges rather than uniformly distributed over the entire forest area (McWilliam et al., 2010b). Forest edges may also reduce animal-mediated seed dispersal of non-native plants from gardens into the forest interior.

4.2. Influence of roads

Our finding that the number of non-native plants was higher close to roads can partly be explained by the fact that roads create disturbance, edge structures and new open spaces, and fragment natural areas (Allen et al., 2013). Road verges act as dispersal corridors for non-native species and this facilitates plant invasions (Vakhlamova et al., 2016). Vehicles play an important role for seed dispersal of non-native plants (Von der Lippe et al., 2005), and the magnitude of this effect increases with increasing cover of traffic infrastructure around the study sites.

4.3. Influence of gardens

In the region of Lugano, the high richness and large abundance of non-native plant species in sites surrounded by a large percentage cover of garden can be explained by the huge variety of ornamental exotic plants that make gardens to important sources for non-native plant species in the wild (Marco et al., 2008). In England, 70% of the garden flora is non-native and domestic gardens are assumed to constitute the largest source of non-native plants in urban and suburban regions (Smith et al., 2006).

Shrub and tree species were the most abundant non-native plant species recorded in the ground vegetation and in the shrub layer in both Swiss regions. There are several potential explanations for this finding. Non-native shrub and tree species are more frequently cultivated in gardens than non-native herbaceous plants (Smith et al., 2006). These shrubs and trees produce larger amounts of biomass that are often illegally dumped as garden waste in forests (Rusterholz et al., 2012), and their seeds are often dispersed by birds, which may facilitate their spread into nearby-situated forests. In fact, illegal garden waste deposits (found in 80% of all near-settlement sites in the present study; L. Gaggini, unpubl.) and bird-mediated seed dispersal may play an important role in the establishment of non-native species in forests (Rusterholz et al., 2012). In this context, it is important to note that most of the non-native species recorded in far-from-settlement sites have seeds, which are dispersed by birds (e.g. *Prunus laurocerasus, Trachycarpus fortunei*) or wind (e.g. *Acer pseudoplatanus* var. *purpurascens*, *Fraxinus potamophila*).

4.4. Regional differences

Our results revealed regional differences, although landscape structure around near-settlement and control sites did not differ between the two regions (data not shown). In the region of Lugano, total species richness in the ground vegetation and shrub layer of deciduous forests was 1.5 times higher than that in the region of

Basel. Non-native species richness, however, was twice as high in the ground vegetation, and three times higher in the shrub layer in the region of Lugano than those in the region of Basel. The higher species richness of non-native plants recorded in the region of Lugano is characteristic for the Southern part of Switzerland (Schönenberger et al., 2014). Forests in the region of Lugano have been exposed to the invasion of non-native plants for a longer period than forests in the region of Basel, where most invasion processes are still in an early phase. This regional difference can be explained by the warmer climate with milder winters in Southern Switzerland, which facilitates the establishment of garden and horticultural plants in forests (Walther et al., 2001). Mild winters are in fact one of the main factors causing changes in plant species composition in forests (Carraro et al., 1999). Private gardens in Southern Switzerland tend to harbour a higher number of non-native species than gardens in Northwestern Switzerland, also reflecting the longer history of introductions of non-native plant species to Southern Switzerland (Schröter, 1936). This could further explain the more advanced invasion process in forests of Southern Switzerland. In contrast to our expectations of a natural expansion of plant species from the Mediterranean area to Southern Switzerland, we did not find any species of Mediterranean origin in the sites examined.

Considering the ongoing climate warming, however, also Northern Switzerland could experience an increased frequency of invasion of non-native plants in the near future (Kleinbauer et al., 2010). For this reason, knowledge from the more advanced invasion in Southern Switzerland can provide basic data to develop management action plans for the prevention of plant invasions in Northern regions.

4.5. Conclusions

Urbanization is accelerating worldwide. It is very important to study and understand ongoing processes in near-settlement forests in order to elaborate new management actions. Prevention is one of the most cheap and efficient way to reduce or even avoid invasion. Given the high fragmentation of green areas in urban and suburban regions, we should allow native species that are present in small remnants to expand into the surrounding urban matrix (Doody et al., 2010). Planting more native plants and shrubs in domestic gardens, and thereby replacing ornamental exotic plant species would help to reduce the on-going spread of non-native plants into natural habitats. Furthermore, management of already invaded forest areas should be accompanied by an increased awareness of nurseries, plant sellers and general public about the negative effects of non-native species. McKinney (2002) stressed the need to develop a more ecologically informed public, because people very often cannot identify whether or not a species is native.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.actao.2016.12.008.

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Supplementary material Chapter I

Table S1

Geographical coordinates and elevation of near-settlement and control sites in the regions of Lugano and Basel, Switzerland (S = near-settlement site, C = control site)

Region	Locality	Site	Coordinates [a]		Elevation (m a.s.l.) [a]
Lugano	Breganzona	S	46°00’42’’N	8°55’33’’E	415
Lugano	Breganzona	C	46°01’12’’N	8°55’50’’E	336
Lugano	Arasio	S	45°58’35’’N	8°54’40’’E	477
Lugano	Arasio	C	45°58’25’’N	8°54’35’’E	503
Lugano	Carabbia	S	45°58’17’’N	8°56’21’’E	536
Lugano	Carabbia	C	45°58’03’’N	8°56’13’’E	522
Lugano	Pazzallo	S	45°58’51’’N	8°56’24’’E	454
Lugano	Pazzallo	C	45°58’42’’N	8°56’13’’E	460
Lugano	Lamone	S	46°02’26’’N	8°56’08’’E	319
Lugano	Lamone	C	46°02’34’’N	8°56’27’’E	430
Lugano	Vezia	S	46°01’45’’N	8°56’34’’E	413
Lugano	Vezia	C	46°01’51’’N	8°56’58’’E	439
Lugano	Ponte Capriasca	S	46°03’48’’N	8°56’55’’E	442
Lugano	Ponte Capriasca	C	46°03’52’’N	8°57’02’’E	446
Basel	Biel-Benken	S	47°30’37’’N	7°31’52’’E	351
Basel	Biel-Benken	C	47°30’42’’N	7°32’03’’E	365
Basel	Reinach	S	47°30’00’’N	7°35’21’’E	326
Basel	Reinach	C	47°30’03’’N	7°34’53’’E	366
Basel	Hofstetten	S	47°28’51’’N	7°30’47’’E	515
Basel	Hofstetten	C	47°28’45’’N	7°31’17’’E	494
Basel	Liestal	S	47°28’45’’N	7°43’34’’E	396
Basel	Liestal	C	47°28’47’’N	7°43’18’’E	431
Basel	Therwil	S	47°30’33’’N	7°33’46’’E	313
Basel	Therwil	C	47°30’20’’N	7°33’53’’E	337
Basel	Oberwil	S	47°30’57’’N	7°34’10’’E	349
Basel	Oberwil	C	47°30’43’’N	7°34’22’’E	353
Basel	Pratteln	S	47°30’41’’N	7°41’42’’E	352
Basel	Pratteln	C	47°30’34’’N	7°41’38’’E	367
Basel	Münchenstein	S	47°30’57’’N	7°37’31’’E	366
Basel	Münchenstein	C	47°30’49’’N	7°38’10’’E	387

[a] Data source: http://www.map.geo.admin.ch/

Table S2

Median values and range of soil characteristics in near-settlement and control sites in the regions of Lugano (each $n = 7$) and Basel (each $n = 8$), Switzerland

	LUGANO					**BASEL**					
	Near-settlement site (S)		Control site (C)		Difference between S and C [a]	Near-settlement site (S)		Control site (C)		Difference between S and C [a]	Difference between Lugano and Basel [b]
	Median	Range	Median	Range		Median	Range	Median	Range		
Soil pH	5.4	4.7–6.2	5.5	5.2–6.5	$t = 2.62$, $P = 0.040$	5.9	5.7–6.4	6.0	5.5–6.5	$t = 0.32$, $P = 0.760$	$t = 3.66$, $P = 0.002$
Soil moisture (%)	35	22–45	32	25–60	$t = 0.16$, $P = 0.882$	26	18–40	28	19–33	$t = 0.54$, $P = 0.606$	$t = 3.23$, $P = 0.004$
Soil organic matter content (%)	29	12–35	31	14–46	$t = 1.14$, $P = 0.297$	18	11–33	18	9–32	$t = 1.33$, $P = 0.224$	$t = 2.89$, $P = 0.008$

[a] Results from paired *t*-tests

[b] Results from unpaired *t*-tests

Note: soil pH in the region of Lugano differed significantly between near-settlement and control sites, but not in the region of Basel.

We excluded soil pH from the models to allow a straightforward comparison of the results of the two regions

Table S3

Definition of site and landscape characteristics used in the analyses, assessed using aerial imageries (http://www.map.geo.admin.ch/, scale 1:2'500, accessed 21 October 2014)

Variable	*Definition*
Site characteristics:	
Distance to nearest road	Shortest distance (m) from the centre of the study site to the nearest road
Landscape characteristics:	Cover (in %) of different landscape elements within radii of 100 m and 200 m around the central plot of each study site
Forest cover	Forest and woodlands
Cover of agriculture land	Cropland, meadows and pastures
Cover of traffic infrastructure	Roads, railway trails, parking areas
Cover of buildings	Built-up area – including houses, industrial and rural buildings
Cover of garden	Ornamental gardens
Forest-urban interface	Length (m) of the interface between forest and buildings or ornamental gardens (modified after Radeloff et al. 2005).

Reference:

Radeloff, V.C., Hammer, R.B., Stewart, S.I., Fried, J.S., Holcomb, S.S., McKeefry, J.F., 2005. The wildland urban interface in the United States. *Ecol. Appl.* 15, 799–805.

Table S4

Spearman rank correlation indices for vegetation cover characteristics recorded at the plot level in the regions of Lugano (n = 42) and Basel (n = 48), Switzerland. Only significant values ($P < 0.05$) are shown

	Vegetation cover
Region of Lugano (R = 100 m):	
Litter cover	-0.78
Dead wood	-0.55
Bare ground	-0.33
Region of Basel (R = 100 m):	
Litter cover	-0.83
Dead wood	-0.48

Note: Bare ground cover in the region of Lugano is significantly correlated with vegetation cover, but not in the region of Basel. We excluded bare ground cover from the models to allow a straightforward comparison of the results of the two regions

Table S5

Spearman rank correlation matrices for the assessed site and landscape characteristics on a spatial scale of R = 100 m in localities near Lugano (n = 7) and Basel (n = 8), Switzerland. Only significant values ($P < 0.05$) are shown

	Distance to nearest road	Forest-urban interface	Forest cover	Agriculture land cover	Cover of traffic infrastructure	Cover of buildings	Cover of gardens
Region of Lugano (R = 100 m):							
Distance to nearest road							
Forest-urban interface	-0.62						
Forest cover	0.57						
Agriculture land cover	0.34	-0.79					
Cover of traffic infrastructure	-0.45	0.53	-0.48	-0.50			
Cover of buildings	-0.59	0.94	-0.34	-0.77	0.62		
Cover of garden	-0.56	0.92	-0.43	-0.78	0.69	0.92	
Region of Basel (R = 100 m):							
Distance to nearest road							
Forest-urban interface							
Forest cover							
Agriculture land cover	0.32	-0.79					
Cover of traffic infrastructure	-0.34	0.81	-0.50	-0.71			
Cover of buildings		0.81	-0.43	-0.82	0.81		
Cover of garden		0.82	-0.38	-0.82	0.74	0.97	

Table S6

Summary of linear mixed-effect model (LME) analyses showing the effects of site and landscape characteristics (cover in % of different landscape elements within a radius of 100 m around each study site) on the number of non-native plant individuals and number of non-native species recorded in the ground vegetation (**a**), and in the shrub layer (**b**) in localities near Lugano (near-settlement sites: 7 / control sites: 7) and Basel (8 / 8), Switzerland. Data were analysed at the level of zones (three zones within site) for each region separately

	LUGANO						BASEL					
	Number of non-native plant individuals			Number of non-native plant species			Number of non-native plant individuals			Number of non-native plant species		
	F	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*	*F*	*df*	*P*
(a) Ground vegetation												
Distance to nearest road (m)	3.84	1, 10	0.079	10.51	1, 10	**0.009**	–	–	–	–	–	–
Forest cover (%)	7.31	1, 10	**0.022**	–	–	–	–	–	–	–	–	–
Cover of traffic infrastructure (%)	–	–	–	–	–	–	–	–	–	4.59	1, 12	0.053
Cover of garden (%)	–	–	–	9.81	1, 10	**0.011**	1.13	1, 13	0.308	1.72	1, 12	0.214
(b) Shrub layer												
Distance to nearest road (m)	2.78	1, 10	0.127	2.79	1, 9	0.129	–	–	–	–	–	–
Forest cover (%)	–	–	–	–	–	–	1.57	1, 13	0.232	2.35	1, 13	0.149
Cover of traffic infrastructure (%)	–	–	–	1.03	1, 9	0.336	–	–	–	–	–	–
Cover of garden (%)	8.68	1, 10	**0.015**	9.66	1, 9	**0.013**	–	–	–	–	–	–

– Excluded from the model after step-wise reduction

Table S7

List of plant species recorded in the ground vegetation at plot level in 15 localities in the regions of Lugano and Basel, Switzerland. Species marked with '*' are non-native to Switzerland. Species marked as 'ornamental' were garden forms clearly distinguishable from wild native species

	LUGANO		BASEL	
Species	Near-settlement site	Control site	Near-settlement site	Control site
Abies alba	x		x	x
Acer campestre	x	x	x	x
Acer platanoides			x	x
Acer pseudoplatanus	x	x	x	x
Acer pseud. var. *purpurascens* *				x
Acer spp. (ornamental) *	x			
Aesculus hippocastanum *			x	x
Ailanthus altissima *	x			
Alliaria petiolata	x			
Allium ursinum			x	x
Alnus glutinosa		x		
Anemone nemorosa	x	x	x	x
Arum maculatum			x	x
Aruncus dioicus	x	x		
Bergenia crassifolia *	x			
Brachypodium sylvaticum			x	x
Bromus sterilis		x		
Buxus sempervirens (ornamental) *			x	
Calystegia sepium	x	x		
Cardamine bulbifera	x	x		
Carex flacca			x	
Carex remota			x	
Carex sylvatica		x	x	x
Carpinus betulus			x	x
Caryopteris spp. (ornamental) *			x	x
Castanea sativa	x	x		
Cephalantera longifolia		x		
Chelidonium majus	x			
Circaea lutetiana	x		x	
Clematis vitalba	x			
Cornus mas	x	x		
Cornus sanguinea		x	x	x
Corylus avellana	x	x	x	x
Corylus avellana var. *fuscorubra* *			x	
Crataegus laevigata			x	x
Crataegus monogyna			x	x
Crocus spp. (ornamental) *	x			
Cyclamen purpurascens	x	x		
Deschampsia cespitosa			x	
Diospyros lotus *	x			
Duchesnea indica *	x	x		
Euphorbia dulcis	x	x		
Fagus sylvatica	x	x	x	x

	LUGANO		BASEL	
Species	Near-settlement site	Control site	Near-settlement site	Control site
Festuca altissima				x
Festuca gigantea			x	
Fragaria vesca		x	x	x
Fraxinus excelsior	x	x	x	x
Fraxinus ornus		x		
Fraxinus potamophila *	x	x		
Galeopsis tetrahit	x	x	x	x
Galium aparine			x	x
Galium odoratum				x
Galium verum		x		
Geranium robertianum	x		x	
Geum urbanum	x	x	x	x
Glechoma hederacea	x		x	x
Hedera helix	x	x	x	x
Helleborus niger	x			
Helleborus viridis		x		
Hepatica nobilis	x			
Ilex aquifolium	x	x	x	
Impatiens parviflora *	x	x		
Kerria japonica *	x			x
Laburnum anagyroides		x		
Lamium album		x		
Lamium galeobdolon			x	x
Lamium galeobdolon subsp. *argentatum* *	x	x	x	x
Lamium galeobdolon subsp. *flavidum* (*) [a]	x	x	x	x
Lamium maculatum			x	x
Lamium purpureum	x	x		
Lapsana communis	x			
Lathyrus pratensis		x		
Laurus nobilis	x	x		
Ligustrum japonicum *			x	
Ligustrum vulgare	x	x	x	x
Liliaceae (ornamental) *	x			
Listera ovata		x		
Lonicera alpigena		x		
Lonicera periclymenum	x	x		x
Lonicera pileata *	x		x	
Lonicera xylosteum	x	x	x	x
Luzula luzuloides	x		x	
Luzula nivea	x	x		
Mahonia aquifolium *		x		
Maianthemum bifolium	x	x		
Mercurialis perennis	x		x	
Oxalis acetosella	x	x		
Paris quadrifolia			x	x
Petasites hybridus (ornamental) *		x		
Phyteuma spicatum			x	
Phytolacca americana *	x	x		
Poa nemoralis	x	x		x
Polygonatum multiflorum	x	x		x
Potentilla sterilis				x
Primula acaulis	x			

	LUGANO		BASEL	
Species	Near-settlement site	Control site	Near-settlement site	Control site
Primula elatior	x		x	x
Prunus avium	x	x	x	x
Prunus domestica *	x	x	x	
Prunus laurocerasus *	x	x	x	
Prunus serotina *	x	x		
Prunus spinosa			x	x
Pulmonaria australis		x		
Pyrus pyraster	x			
Quercus petraea	x		x	x
Quercus robur	x	x	x	x
Ranunculus auricomus			x	x
Ranunculus ficaria	x		x	x
Rhamnus caroliniana *		x		
Robinia pseudoacacia *	x			
Rosa arvensis	x		x	x
Rubus spp.	x	x	x	x
Rubus spp. (ornamental) *	x			
Rubus ulmifolius			x	
Ruscus aculeatus	x	x		
Sambucus nigra	x	x		
Sambucus racemosa		x		
Sorbus aria			x	
Stellaria media	x			
Taraxacum officinale		x		
Tilia platyphyllos	x	x	x	x
Tilia spp. (ornamental) *	x			
Trachycarpus fortunei *	x	x		
Ulmus minor	x	x		
Urtica dioica				x
Veronica beccabunga			x	
Viburnum lantana			x	x
Viburnum rhytidophyllum *	x			
Vicia sepium			x	
Vinca major	x			
Vinca minor	x	x		
Viola hirta		x		
Viola reichenbachiana	x	x	x	x
Vitis sylvestris *	x			
Unknown 1		x		
Unknown 2		x		

[a] Plant species is native to Southern Switzerland (Lugano), but non-native to Northwestern Switzerland (Basel)

Table S8

List and characteristics of non-native plant species recorded in the study sites in the regions of Lugano and Basel, Switzerland. The list comprises species found at the plot level or at the level of zones, both in the ground vegetation and in the shrub layer

Species name	LUGANO	BASEL	Status [a]	Life form	Frequency [b]	Origin [c]
Acer pseudoplatanus var. *purpurascens*		x	non-native	deciduous tree	2	
Acer spp. (ornamental)	x		non-native	deciduous tree	2	
Aesculus hippocastanum		x	non-native	deciduous tree	2	South-East Europe
Ailanthus altissima	x		invasive	deciduous tree	1	Asia (China)
Berberis spp. (ornamental)		x	non-native	shrub	1	
Bergenia crassifolia	x		non-native	herbaceous plant	1	Asia
Buxus sempervirens (ornamental)		x	non-native	evergreen shrub	1	
Campanula trachelium (ornamental)		x	non-native	herbaceous plant	1	
Caryopteris spp. (ornamental)		x	non-native	herbaceous plant	2	
Corylus avellana var. *fuscorubra*		x	non-native	shrub	1	
Crocus spp. (ornamental)	x		non-native	herbaceous plant	1	
Diospyros lotus	x		non-native	shrub	1	Asia
Duchesnea indica	x		non-native	herbaceous plant	2	South-East Asia
Elaeagnus pungens	x		non-native	shrub	1	East Asia
Fraxinus potamophila	x		non-native	deciduous tree	2	
Geranium robertianum subsp. *purpureum*		x	non-native	herbaceous plant	1	Mediterranean
Hedera helix (ornamental)		x	non-native	climbing woody plant	1	
Hemerocallis spp. (ornamental)	x		non-native	herbaceous plant	1	
Impatiens balfourii	x		watch list	herbaceous plant	1	Himalaya (Asia)
Impatiens parviflora	x		non-native	herbaceous plant	4	Central and East Asia
Kerria japonica	x	x	non-native	shrub	2	Asia
Lamium galeobdolon subsp. *argentatum*	x	x	non-native	herbaceous plant	8	Europe, culture form
Lamium galeobdolon subsp. *flavidum* [d]	x	x	non-native	herbaceous plant	7	Native in Southern Switzerland
Ligustrum japonicum		x	non-native	evergreen shrub	1	Japan and East Asia

Species name	LUGANO	BASEL	Status [a]	Life form	Frequency [b]	Origin [c]
Liliaceae (ornamental)	x		non-native	herbaceous plant	2	
Lonicera pileata	x	x	non-native	evergreen shrub	4	Asia (China)
Mahonia aquifolium	x		non-native	evergreen shrub	1	North America
Parthenocissus tricuspidata		x	non-native	deciduous woody vine	1	East Asia
Petasites hybridus (ornamental)	x		non-native	herbaceous plant	1	
Petasites spp. (ornamental)	x		non-native	herbaceous plant	1	
Phytolacca americana	x		watch list	herbaceous plant	2	North America
Prunus domestica	x	x	non-native	deciduous tree	3	South-West Asia
Prunus laurocerasus	x	x	invasive	evergreen shrub / tree	10	South Europe / South-West Asia
Prunus serotina	x		invasive	deciduous tree	3	North America
Rhamnus caroliniana	x		non-native	shrub / tree	1	North America
Robinia pseudoacacia	x		invasive	deciduous tree	4	North America
Rubus spp. (ornamental)	x		non-native	small shrub	2	
Sorbus spp. (ornamental)	x		non-native	deciduous tree	2	
Tilia spp. (ornamental)	x		non-native	deciduous tree	1	South-East Europa / West Asia
Trachycarpus fortunei	x		invasive	palm	8	East Asia
Viburnum rhytidophyllum	x		non-native	evergreen shrub	1	East Asia
Vitis sylvestris	x		non-native	liana	1	South-East Europa / West Asia

[a] Assessment of the invasion status, http://www.infoflora.ch/ (accessed 19.1.2015)

[b] Number of study sites where the species occurs, out of a total of 30 sites

[c] Following Schönenberger, N., Röthlisberger, J., Carraro, G., 2014. La flora esotica del Cantone Ticino (Svizzera). Bollettino della Società Ticinese di Scienze Naturali. 102, 13–30

[d] Plant species is native to Southern Switzerland (Lugano), but non-native to Northwestern Switzerland (Basel)

Table S9

Number of non-native plant individuals and number of non-native species (means ± SE) recorded in the three zones of near-settlement and control sites in the ground vegetation (**a**), and in the shrub layer (**b**) in the regions of Lugano (each $n = 7$) and Basel (each $n = 8$), Switzerland

	LUGANO				BASEL			
	Number of non-native plant individuals		Number of non-native plant species		Number of non-native plant individuals		Number of non-native plant species	
	Near-settlement sites	Control sites	Near-settlement sites	Control sites	Near-settlement sites	Control sites	Near-settlement sites	Control sites
(a) Ground vegetation								
Zone 1	177.7 ± 66.2	26.0 ± 17.0	5.7 ± 0.6	2.3 ± 0.6	96.3 ± 41.9	21.6 ± 19.8	2.8 ± 0.6	0.9 ± 0.4
Zone 2	102.6 ± 67.2	11.4 ± 5.8	3.6 ± 0.6	1.3 ± 0.4	64.0 ± 42.0	19.3 ± 19.0	1.1 ± 0.2	0.5 ± 0.3
Zone 3*	49.6 ± 31.5	3.9 ± 1.7	2.1 ± 0.3	0.9 ± 0.3	23.9 ± 22.3	22.5 ± 22.5	0.2 ± 0.1	0.1 ± 0.1
(b) Shrub layer								
Zone 1	47.3 ± 12.2	12.1 ± 3.6	4.1 ± 0.7	2.6 ± 0.4	4.3 ± 1.6	2.1 ± 1.8	1.0 ± 0.4	0.4 ± 0.2
Zone 2	35.1 ± 7.5	11.7 ± 5.7	3.3 ± 0.6	2.0 ± 0.5	3.3 ± 2.3	0.8 ± 0.8	0.6 ± 0.4	0.1 ± 0.1
Zone 3*	21.9 ± 9.9	5.2 ± 2.2	1.7 ± 0.3	1.3 ± 0.2	0.3 ± 0.3	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0

* Data of zone 3 were related to the size of zones 1 and 2

Fig. S1

Photographs showing two pairs of study sites in the regions of Lugano and Basel, Switzerland: near-settlement (**a**) and control site (**b**) in Pazzallo, near to Lugano; near-settlement (**c**) and control site (**d**) in Pratteln, near to Basel. Near-settlement sites were located adjacent to settlements, whereas control sites were located far from settlements, in proximity of a meadow and with no buildings in the surroundings. Photo credit: L. Gaggini

(a)

(b)

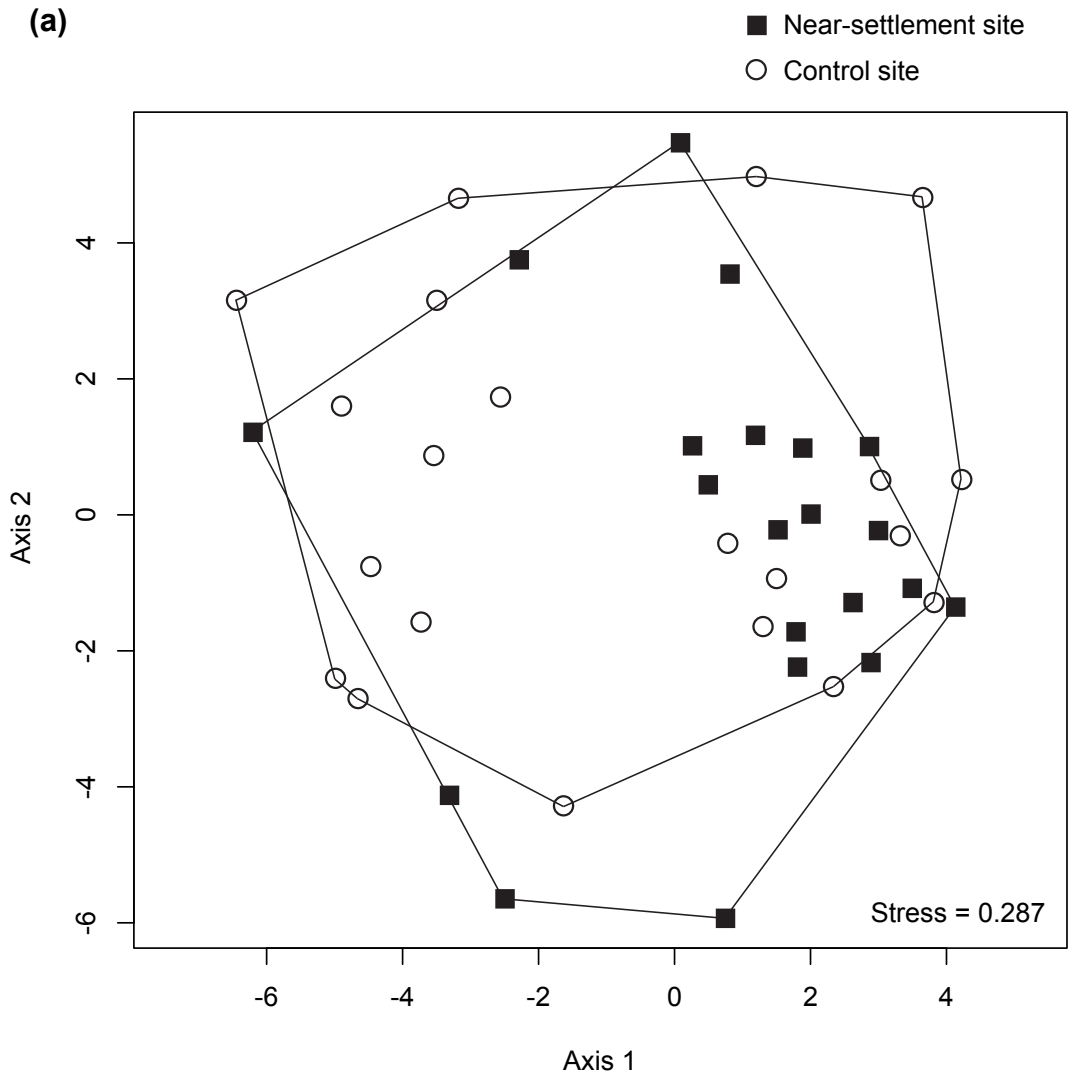
(c)

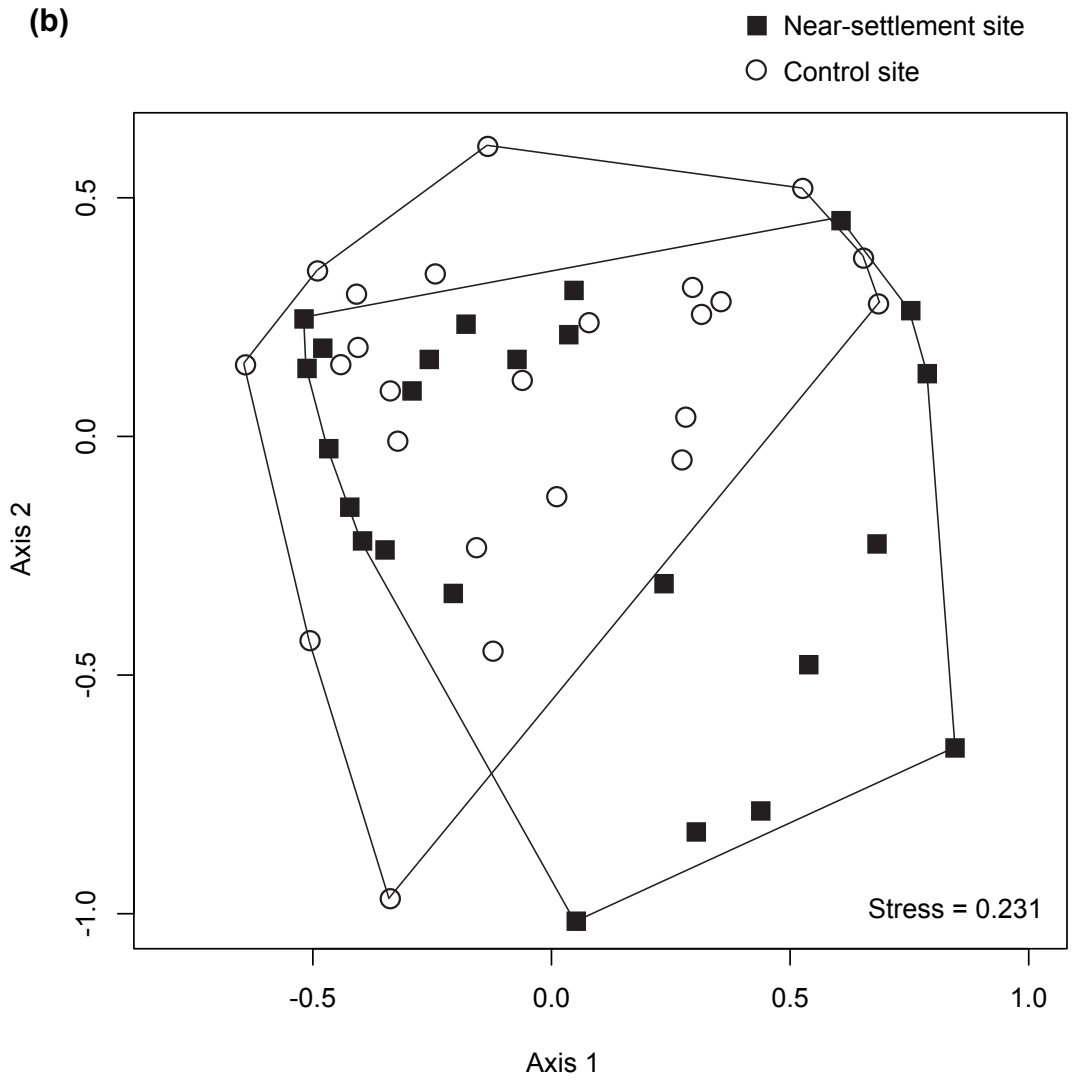
(d)

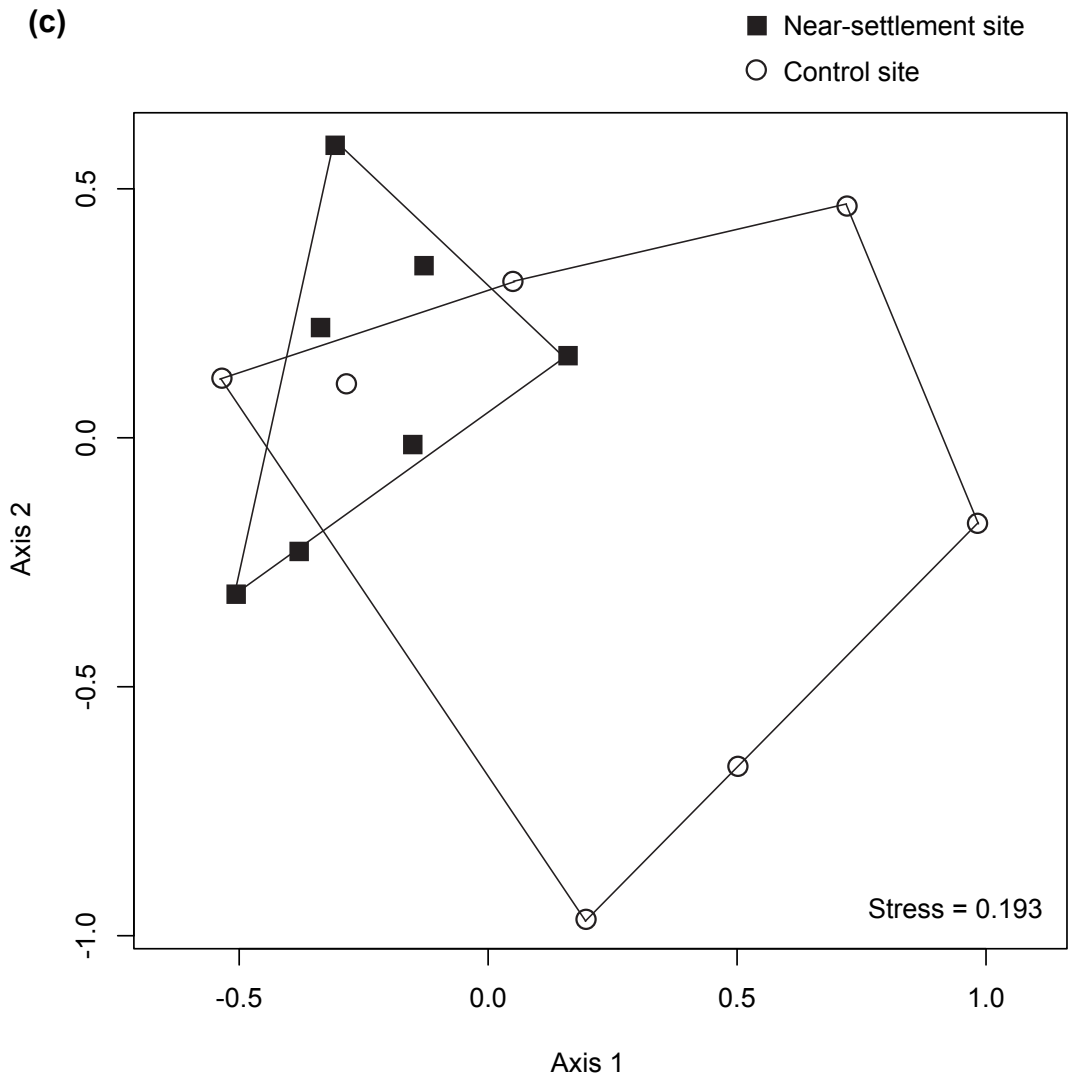
Fig. S2

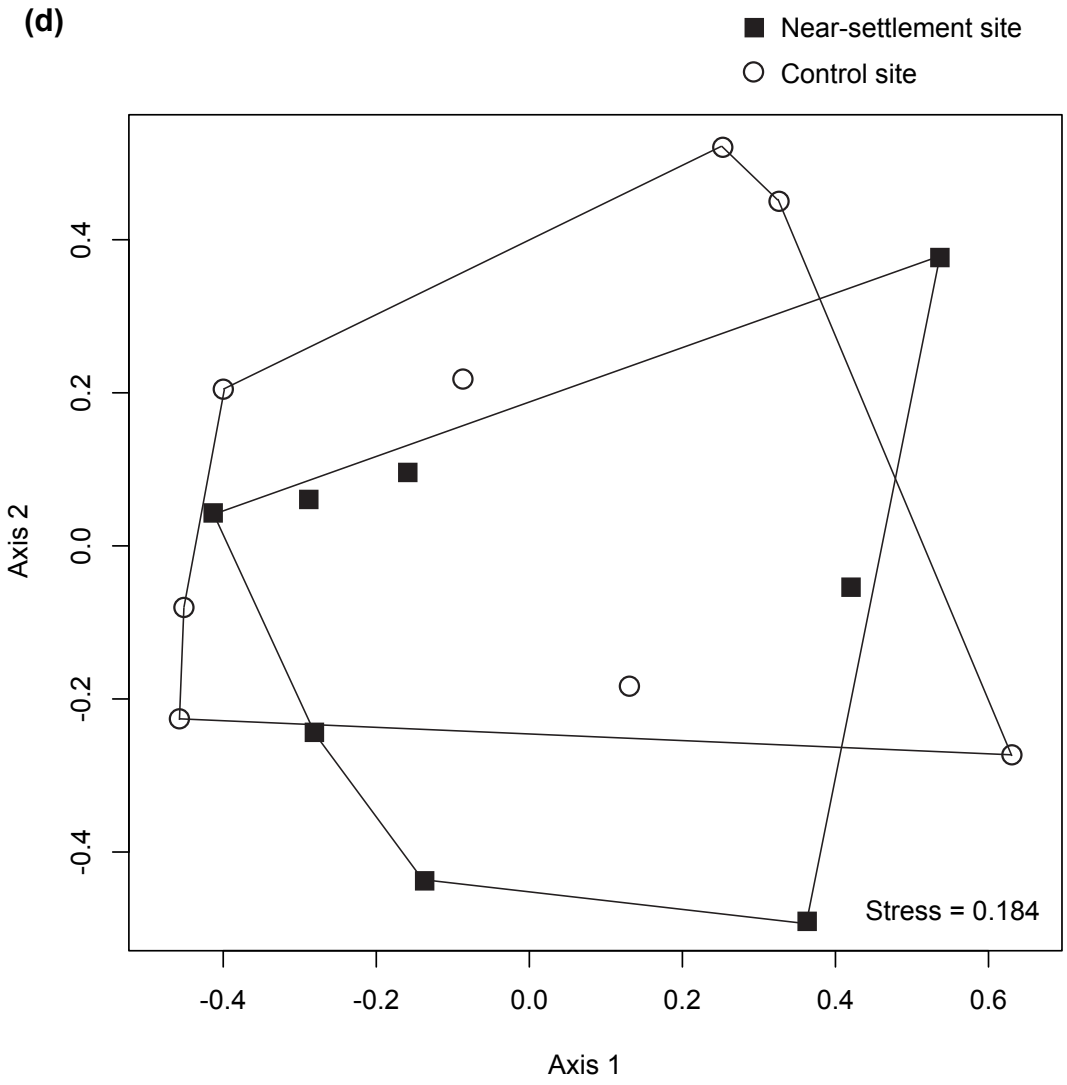
Non-metric multidimensional scaling (NMDS) ordination diagrams based on Bray-Curtis dissimilarities in plant species composition recorded in near-settlement and control sites in the regions of Lugano (**a**, **c**) and Basel (**b**, **d**), Switzerland. The analysis was conducted at two different levels: at the subplot level (4 m^2; a, b) and at the site level (three subplots pooled, 12 m^2; c, d)

(**a**) Lugano (4 m^2-level): NMDS with *envfit*, proximity to settlements: $R^2 = 0.050$, $P = 0.113$
(**b**) Basel (4 m^2-level): NMDS with *envfit*, proximity to settlements: $R^2 = 0.041$, $P = 0.137$
(**c**) Lugano (12 m^2-level): NMDS with *envfit*, proximity to settlements: $R^2 = 0.179$, $P = 0.094$
(**d**) Basel (12 m^2-level): NMDS with *envfit*, proximity to settlements: $R^2 = 0.030$, $P = 0.684$




(b)
Near-settlement site
Control site
Axis 2
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0
Axis 1
Stress = 0.231


(c)
Near-settlement site
Control site
Axis 2
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0
Axis 1
Stress = 0.193


(d)
Near-settlement site
Control site
Stress = 0.184
Axis 1
Axis 2
-0.4
-0.2
0.0
0.2
0.4
0.6
-0.4
-0.2
0.0
0.2
0.4

Chapter II

The invasive plant *Impatiens glandulifera* affects soil fungal diversity and the bacterial community in forests

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The invasive plant *Impatiens glandulifera* affects soil fungal diversity and the bacterial community in forests

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ABSTRACT

Invasive plants may severely impact native biodiversity and ecosystem functioning. We examined potential effects of the invasive annual plant *Impatiens glandulifera* Royle on soil fungal and bacterial communities in temperate forests. Using a space-for-time approach, we established 72 plots in forest areas invaded by *I. glandulifera* and in forest areas, which were not yet invaded, equally distributed over three coniferous and three deciduous forests. In each plot, we determined plant species richness and abundance in the above-ground vegetation as well as the diversity and composition of the soil fungal community using T-RFLP analysis. Biolog Ecoplates were used to assess the activity of soil bacteria. The invasion of *I. glandulifera* caused significant shifts in plant species composition. Invaded plots were characterized by a higher diversity and an altered composition of the soil fungal community and by a lower soil bacterial activity in late spring. Carbon substrate utilization patterns of soil bacteria were also changed in invaded plots. Our experiment shows that *I. glandulifera* can modify soil fungal and bacterial communities, indicating an indirect effect of altered soil properties induced by the invasive plant, combined with the release of allelopathic compounds into the soil.

1. Introduction

The invasion of non-native species into natural habitats is considered as a major threat to native biodiversity (Pimentel et al., 2005; Pejchar and Mooney, 2009). Non-native species are key drivers of human-caused global environmental change and have the potential to affect ecosystems by altering native species diversity, community structure and interactions among organisms (Vilà et al., 2011; Pyšek et al., 2012; Stoll et al., 2012).

There is increasing evidence that "above-ground" biodiversity positively affects ecosystem functioning and services (Zavaleta et al., 2010; Isbell et al., 2011). In contrast, there is a gap in the knowledge of the role of "below-ground" diversity for ecosystem functioning. For example, Bardgett and van der Putten (2014) underlined the important role of the diversity of below-ground communities for shaping above-ground diversity and emphasized that the composition of the below-ground community might be more important than its species diversity for ecosystem functioning.

Soil fungi can be classified to be mutualistic, saprophytic, endophytic or pathogenic (Danielsen et al., 2012; Dighton, 2016). Soil fungi are a key component of below-ground communities and are involved in a variety of microbiological and ecological processes influencing soil fertility, decomposition, cycling of minerals and organic matter (Itoo and Reshi, 2013). Among them, mycorrhizal fungi, constituting a mutualistic symbiosis between soil fungi and plants, represent the main part of soil fungi in terms of biomass (Nehls, 2008) and play a crucial role for the establishment, survival and growth of vascular plants including trees and for the regeneration of forests (Courty et al., 2010; Simard et al., 2012).

Invasive plants are able to alter soil properties and soil processes (see reviews in Ehrenfeld, 2003; Raizada et al., 2008; Weidenhamer and Callaway, 2010). There is increasing evidence that invasive plants can affect species richness and composition of soil fungal communities (Callaway et al., 2004; Wolfe and Klironomos, 2005; Rodríguez-Echeverría and Traveset, 2015) and disrupt symbiotic associations between soil fungi and host plants (Ruckli et al., 2016). Hawkes et al. (2006) pointed out that alteration of the mycorrhizal fungal community may also be an effective mechanism through which plant invaders can affect ecosystem functions.

Beside soil fungal communities, alien plant species can also alter the activity and structure of soil bacterial communities (Kourtev et al., 2002; Lorenzo et al., 2013; Qin et al., 2014). Soil bacteria, together

Abbreviations: AMF, arbuscular mycorrhizal fungi; EMF, ectomycorrhizal fungi; LME, linear mixed-effect model; TRF, terminal restriction fragment; T-RFLP, terminal restriction fragment length polymorphism; OTU, operational taxonomic unit

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0929-1393/

with soil fungi, play a key role in energy flow, nutrient cycling and organic matter turnover in ecosystems (Bauhus and Khanna, 1999). Because bacteria are very sensitive to changes in the environment, alterations in soil bacterial communities may precede and reflect changes in the whole ecosystem (Stefanowicz, 2006).

In our study, *Impatiens glandulifera* Royle (Himalayan balsam), an herbaceous annual plant belonging to the family Balsaminaceae, was chosen as focal invasive species. It is native in the western Himalaya and was introduced as garden ornamental plant to Europe and North America in the middle of the 19th century (Beerling and Perrins, 1993). It became naturalized and invasive in riparian and disturbed habitats (Hejda and Pyšek, 2006). In the last decades, *I. glandulifera* has increasingly invaded deciduous and coniferous forests, owing to natural and man-related disturbances (Nobis, 2008; Wagner et al., 2017). It is classified as one of the 100 worst alien species in Europe (DAISIE, 2009). *I. glandulifera* can cause slight changes in plant species richness and shifts in plant species composition in riparian habitats (Hejda and Pyšek, 2006; Diekmann et al., 2016), besides promoting soil erosion along riparian zones (Greenwood and Kuhn, 2014). In deciduous forests, *I. glandulifera* alters soil characteristics (Ruckli et al., 2013, 2014a; Rusterholz et al., 2014), gastropod communities (Ruckli et al., 2013) and soil invertebrate communities (Tanner et al., 2013; Rusterholz et al., 2014). Furthermore, this invasive plant reduces species richness and alters the composition of the soil seed bank with a delay of several years (Rusterholz et al., 2017). Leaves and roots of *I. glandulifera* contain an allelopathic compound (2-methoxy-1,4-naphthoquinone), which is released into the soil, having strong inhibitory effects on the growth of mycorrhizal fungi and on the germination of several native herbs (Ruckli et al., 2014b). Furthermore, forests invaded by *I. glandulifera* show a reduced mycorrhizal colonization of roots and thus a reduced survival of tree saplings (Tanner and Gange, 2013; Ruckli et al., 2014a, 2016; Pattison et al., 2016). These findings support the "novel weapons hypothesis", which assumes that some invasive plant species produce secondary metabolites (allelochemicals) that are novel in their non-native ranges and that this novelty provides advantages to the invasive plant as it interacts with native plants, microbes or generalist herbivores (Callaway and Ridenour, 2004). The lower level of mycorrhizal colonization of native plant roots in invaded soils suggests that *I. glandulifera* can effectively change the mycorrhizal fungal community, but studies providing direct evidence for alteration in diversity or composition of soil fungal communities are so far lacking.

The majority of studies investigating the impact of invasive plants on soil fungal communities were carried out in grassland ecosystems (e.g. Hawkes et al., 2006; Mummey and Rillig, 2006; Zubek et al., 2016). However, some studies examined the effects of invasive plants on mycorrhizal fungi in forests situated in Asia (Niu et al., 2007) and North-America (e.g. Yamasaki et al., 1998; Stinson et al., 2006; Callaway et al., 2008; Wolfe et al., 2008; Barto et al., 2011). For example, Barto et al. (2011) showed that the invasive *Alliaria petiolata* does not change mycorrhizal fungal richness but alters their species composition in the community. However, knowledge about the impact of invasive plants on soil fungi in Central European forests is still limited.

The aim of this study was to examine the impact of *I. glandulifera* on the diversity of soil fungi and soil bacterial communities in deciduous forests dominated by *Fagus sylvatica* L. and in coniferous forests dominated by *Picea abies* (L.) H. Karst. or *Abies alba* Mill., the most abundant forest types in Switzerland and Central Europe. We established plots in forest areas with dense stands of *I. glandulifera* and plots in forest areas that were not yet colonized by the invasive plant to compare soil fungal and soil bacterial community. We applied terminal restriction fragment length polymorphism analysis (T-RFLP; see review in Thies, 2007; examples in Mummey and Rillig, 2006; Barto et al., 2011), a well established method to assess the genetic diversity and composition of soil fungal communities and used Biolog Ecoplates to estimate the activity of soil bacterial communities (Stefanowicz, 2006; Lorenzo et al., 2013; Qin et al., 2014). In particular, we tested the following hypotheses: The invasion of *I. glandulifera* 1) reduces species richness of soil fungi and 2) causes shifts in the composition of the fungal community. *I. glandulifera* 3) increases the activity and 4) alters the composition of the soil bacterial community.

2. Material and methods

2.1. Study sites and design of the survey

The experiment was conducted in six study sites situated in a forest 15 km south of Basel, Northwestern Switzerland (47°26' N, 7°33' E). This region has a mean annual temperature of 9.4 °C and an annual precipitation of 947 mm (MeteoSwiss, 2016). The study sites were located within an area of 1.9 km × 1.0 km, with a distance of 100–840 m (mean 600 m) between each other. Elevation of the study sites ranged from 378 to 433 m a.s.l. Three study sites were located in stands dominated by *Picea abies* or *Abies alba* (hereafter referred to as 'coniferous forests'), the other three in stands dominated by *Fagus sylvatica* (hereafter referred to as 'deciduous forests'). The forest was affected by the windstorm Lothar in 1999. *I. glandulifera* started to invade several sites shortly after the storm in spring 2000. In spring 2015 (15 years after the beginning of the invasion), we selected a 15 m × 25 m area invaded by *I. glandulifera* (hereafter referred to as "invaded area") in each study site. An area of the same size which was not yet colonized by the alien plant (hereafter referred to as "uninvaded area") was chosen in proximity to the corresponding invaded one (mean distance between invaded and uninvaded area 50 m, range 30–80 m). Pairs of invaded and uninvaded areas did not differ in elevation, inclination, exposition, forest type and forest management (Table S1 – Electronic Supplementary material). In each invaded area, we installed six randomly chosen 1 m × 1 m plots which had a similar cover of *I. glandulifera* (Table S2). Similarly, six 1 m × 1 m plots with an equal spatial distribution were installed in the corresponding uninvaded areas. This resulted in a total of 72 plots (six plots x two invasion states [invaded and uninvaded areas] x six sites [three in coniferous and three in deciduous forests]). Thus, our experimental design followed a space-for-time substitution approach (Pickett, 1989; Diekmann et al., 2016). The uninvaded areas are assumed to represent the situation prior to the invasion of *I. glandulifera*. In 2017, two years after the present study, two of the formerly uninvaded areas were partially colonized by *I. glandulifera*, showing that both our invaded and uninvaded areas provide suitable habitat for the invasive species.

2.2. Data collection

To assess the seasonal impact of *I. glandulifera* on diversity and composition of the soil fungal community, we collected soil samples in each plot four times in the course of the vegetation period: 2/3 June (juvenile plants), 27/28 July (flowering plants), 29/30 September (fruiting plants) and 1/2 December 2015 (partly decomposed plants). At each sampling occasion, three randomly chosen soil samples were collected in each 1 m × 1 m plot using a soil corer (depth 5 cm, diameter 5.05 cm, volume 100 cm^3). The three samples of a plot were pooled and mixed, resulting in a total of 288 samples (72 plots x four sampling occasions). Soil samples were transported on ice to the laboratory, where they were sieved (mesh size 2 mm) and stored at −80 °C for further analyses. At each sampling occasion, the density, height and biomass of *I. glandulifera* were assessed in additional plots of 20 cm × 20 cm adjacent to the invaded plots.

The cover of each plant species belonging to the above-ground vegetation (herbs and woody plants up to a height of 50 cm) was visually estimated using the Domin scale (Mueller-Dombois and Ellenberg, 2002) in each 1 m × 1 m plot at the end of May 2016. In addition, total cover of ground vegetation, mosses, leaf litter, dead wood and bare ground were visually estimated (accuracy 5%) and the mean and

maximal height of the ground vegetation were measured. Plant surveys were repeated in the mid of September 2016 to complete plant species lists. The recorded plants were classified according to Lauber et al. (2012). Six leaf litter samples (20 cm × 20 cm) were collected adjacent to each plot in September 2016 and their biomass was assessed (dry weight).

To characterize the forest community of the invaded and uninvaded areas in the six study sites, we subdivided each area into five 5 m × 15 m stripes. In each of the five stripes, all plant species belonging to the shrub layer (woody plants, with a height between 50 cm and 5 m) and to the tree layer (height > 5 m) were recorded and the number of individuals for each species were counted in September 2016 (Table S1). We also estimated total vegetation cover of the above-ground vegetation, shrub layer and tree layer, as well as the cover of leaf litter, dead wood and bare ground and that of *I. glandulifera* (accuracy 5%) in each stripe. The girth (cm) of each individual tree was measured at breast height. Canopy closure of the invaded and uninvaded areas was assessed using ten photographs regularly distributed in each study area and determined with the pixel counting function of Adobe Photoshop (version 10.0.1).

2.3. Soil characteristics

Soil samples collected in each plot on the four sampling occasions were dried for 48 h at 60 °C. Soil moisture (%) was determined using the fresh weight to dry weight ratio. Soil pH was assessed in distilled water (1:2.5 soil:water) (Allen, 1989). Total soil organic matter content (%) was determined as loss-on-ignition of oven-dried soil at 750 °C for 16 h (Allen, 1989) and total soil organic nitrogen content (%) was assessed using the standard method of Kjeldahl (Bremner, 1965). Finally, total phosphorus content (μg PO_4^{3-}/g) was determined using ignited soil (500 °C, 4 h), HCl extraction and with the molybdenum blue method (Sparks et al., 1996).

Using 48 randomly chosen soil samples we examined the correlations between total soil organic matter, total soil organic nitrogen and total phosphorous content. Total soil organic nitrogen ($r_s = 0.91$, n = 48, $P < 0.001$) and total phosphorus content ($r_s = 0.51$, n = 48, $P < 0.001$) were significantly correlated with total soil organic matter content, supporting a previous finding obtained in the same forest area (H.-P. Rusterholz, unpublished). As a result of these intercorrelations, we did not analyse total soil organic nitrogen and total phosphorus content in the remaining samples.

2.4. Soil fungal community profiles (T-RFLP)

Soil fungal community profiles were assessed using the T-RFLP method (terminal restriction fragment length polymorphism; Thies, 2007). DNA of the soil samples was extracted using NucleoSpin Soil kit (Macherey-Nagel, Oensingen, Switzerland). For soil fungal analyses, the internal transcribed spacer (ITS) region of fungal DNA was amplified using the fungal specific primer pair ITS1-F/ITS4 (Gardes and Bruns, 1993). Primer ITS1-F was fluorescently labelled at the 5'-end (FAM). PCR reactions (50 μL) consisted of 10 μL of template DNA (10–15 ng/μL), 25 μL Master mix (HotStarTaq Master Mix Kit, Qiagen), 5 μL Primer ITS1-F (10 μM), 5 μL Primer ITS4 (10 μM), 1 μL BSA (1 μg/μL) and 4 μL sterile water. Amplification was achieved in an Eppendorf Mastercycler Pro (Vaudaux-Eppendorf AG, Schönenbuch, Switzerland) under the following conditions: initial 15 min heat activation step at 95 °C, followed by 35 amplification cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min. All reactions were done in triplicate. PCR products were cleaned-up using NucleoSpin gDNA Clean-up kit (Macherey-Nagel, Oensingen, Switzerland). Restriction digestion was performed using TaqI enzyme: for each sample 10 μL of PCR product (130–150 ng/μL), 4 μL of 10× Buffer TaqI (TaqI, Thermo Fisher Scientific Inc.), 4 μL of TaqI (TaqI, Thermo Fisher Scientific Inc.) and 24 μL of sterile water were mixed and incubated at 65 °C for 16 h. The reaction was stopped by adding 1.8 μL 0.5 M EDTA in each sample. The presence of successfully digested products was checked by analysing 5 μL restriction products on a 2% agarose gel, stained with ethidium bromide and visualized under UV light. Samples were prepared as GeneScan samples (1.5 μL DNA sample; 1.5 μL GeneScan 500 LIZ size standard; 17 μL Hi-Di Formamide, Applied Biosystems, Life Technologies) and T-RFLP profiles were produced by sequencing of the digests by Macrogen Inc. (Amsterdam, The Netherlands). Out of a total of 288 soil samples, 38 T-RFLP analyses failed (13%) due to very high amount of organic matter in the soil samples.

The size and the relative abundance of terminal restriction fragments (TRFs) were quantified using Peak Scanner software (version 1.0, Applied Biosystems, Inc.). Data for the four sampling occasions were analysed separately. First, to determine the extent of variation of the bp length, size and areas of the peaks in the T-RFLP analysis, four samples were analysed in duplicate. The obtained average standard deviation of duplicate TRF size was 0.03, an error that can be considered small if compared with previous literature (Smith et al., 2005). The variation in duplicate percentage peak areas was of 19.7%, a larger error than what found in previous literature (Osborn et al., 2000; Smith et al., 2005). TRFs with a size ranging from 50.0 bp to 500 bp were considered in the analyses. To avoid possible background noise, only fragments with a signal above 1% of the sum of all peak areas were included in the analyses (Li et al., 2007; Yang et al., 2016). TRFs in different profiles that differed in size by less than 1.0 bp were considered as the same (Smith et al., 2005; Barto et al., 2011).

2.5. Bacterial community analysis (Ecoplates)

Ecoplates assess physiological profiles of bacterial communities. Due to the pronounced seasonal activity of microbial organisms in forest soils with peaks in spring and autumn (Rastin et al., 1988; Boerner et al., 2005), we evaluated the potential seasonal influence of *I. glandulifera* on the activity and metabolic diversity of soil bacterial communities in late spring (2/3 June) and late autumn (1/2 December 2015). In 2015, the phenology of the above-ground vegetation was delayed by at least 20 days due to an extreme period of drought in April. We assessed the activity and metabolic diversity of soil bacterial communities by using Biolog Ecoplates™ (Biolog Inc., Hayward, CA, USA). Each plate contains three replicates of 31 different single C sources and a blank as a control. The 31 C sources represent six classes of organic compounds: amino acids, amines, carbohydrates, carboxylic acids, polymers and miscellaneous. Soil samples were thawed at 4 °C. A study using samples from the same forest soil type revealed that freezing and thawing had no effect on soil bacterial biomass and the activity of soil enzymes (Kissling et al., 2009).

Plates were prepared following Classen et al. (2003) and Niklińska et al. (2005). The equivalent of 4 g dry weight of moist soil (corrected for the moisture content of each sample) was shaken for 60 min in 40 mL NaCl solution (0.9%) and allowed to settle for 45 min at 4 °C. A 1 mL aliquot of the supernatant was diluted in 9 mL NaCl (0.9%). This dilution was repeated a second time to achieve a final dilution of 10^{-3}. 120 μL of the 10^{-3} diluted solution were inoculated in each well. Ecoplates were incubated at 25 °C in the dark. The optical density at 590 nm (colour development plus turbidity) and 750 nm (turbidity only) was measured directly after inoculation and then every 24 h for 7 days. Plates were read on a Synergy HTX Multi-mode Reader (Biotek Instruments GmbH, Luzern, Switzerland). To minimize the risk of contamination, all solutions used were prepared with sterile water and the preparation of the plates was done under a laminar-flow hood. Microbial community activity was expressed as average well colour development (AWCD) corrected by the diffusion of the wells (absorbance at 750 nm) and the colour development in the control well, according to Classen et al. (2003).

2.6. Data analyses

Statistical analyses were performed in R (R Foundation for Statistical Computing 2014, version 3.1.2). Linear mixed-effect models (LME) were used to analyse the effects of invasion status (invaded/uninvaded) and forest type (coniferous/deciduous) on soil properties (moisture, total soil organic matter, pH), separately for each sampling occasion. To avoid pseudoreplication, plot was nested in area, area was nested in study site, and both were included as random factors. Invasion status and forest type were included as fixed factors. This statistical model was used in the subsequent analyses.

Similar LME models with nested design (see above) were applied to assess the effects of invasion status, forest type and plot characteristics on plant species richness and plant species diversity (Shannon index). Ground vegetation cover, soil moisture and soil pH were included as cofactors (soil organic matter content was excluded from all the models because of intercorrelations).

Permutational multivariate analyses of variance (PERMANOVA) were used to test whether the invasion of *I. glandulifera* affects the plant species composition in the ground vegetation (Anderson, 2005). We excluded *I. glandulifera* data for this type of analysis. Amount of leaf litter, soil moisture and soil pH were included as cofactors. All PERMANOVA tests were based on 999 permutations of the untransformed raw data, using the *adonis* function in the *vegan* R-package.

LME models with nested design (see above) were applied to assess whether soil fungal diversity (number of operational taxonomic units, OTUs) was affected by invasion status, forest type and plot characteristics. Ground vegetation cover, number of plant species, amount of leaf litter, soil moisture and soil pH were included as cofactors. This was done separately for all four sampling occasions.

The same PERMANOVA models as described above were used to test for the effects of invasion status, forest type and plot characteristics on fungal species composition. Data with presence/absence of OTUs were used in the analyses, since peak areas resulting from T-RFLP analyses are considered as an inappropriate measure for abundance due to its low reproducibility (Dickie and FitzJohn, 2007; Barto et al., 2011). Analyses were conducted separately for the four sampling occasions.

To assess potential effects of invasion status, forest type and soil characteristics on the total average bacterial carbon substrate utilization (Ecoplates), LME model analyses with nested design (see above) and with maximum likelihood were conducted. Values based on 168 h incubation were used for the analyses, following the suggestion of Preston-Mafham et al. (2002). Soil moisture and soil pH were included as cofactors.

PERMANOVAs were used to analyse the effects of invasion status, forest type and plot characteristics on bacterial utilization patterns of six different carbon substrate classes (Preston-Mafham et al., 2002). These analyses indirectly examine changes in the composition of soil bacterial communities. Analyses were basedon 999 permutations of the untransformed raw data.

All models were stepwise reduced as recommended by Crawley (2007).

3. Results

3.1. Soil characteristics

At all four sampling occasions, soil moisture was 30–70% higher in plots invaded by *I. glandulifera* than in uninvaded plots (Tables 1 and S3). In contrast, total soil organic matter and soil pH did not differ between invaded and uninvaded plots (Table 1). Soil moisture and total soil organic matter differed between coniferous and deciduous forests in July 2015, but not at the other sampling occasions (Table 1).

3.2. Plant species richness and species composition

A total of 65 plant species were recorded in the six study sites (Table S4). In coniferous forests, out of a total of 58 plant species, 47 (81.0%) were found in invaded areas and 38 (65.5%) in uninvaded areas. The corresponding values for deciduous forests were a total of 51 species, 45 (88.2%) in invaded respectively 40 (78.4%) in uninvaded areas. In both forest types, the number of plant species (coniferous forests, invaded: 11.5 ± 1.6, uninvaded: 12.9 ± 1.3; deciduous forests, invaded: 10.2 ± 2.0, uninvaded: 8.3 ± 1.9; means ± SE, n = 18) and Shannon diversity did not differ between invaded and uninvaded plots, but were influenced by soil pH (Table S5). In contrast, plant species composition differed between invaded and uninvaded plots, and also between coniferous and deciduous forest stands (Table S6). Plant species composition was further influenced by soil moisture (Table S6). The significant interaction between forest type and invasion status

Table 1
Summary of linear mixed-effect model (LME) analyses showing the effects of forest type and presence of *Impatiens glandulifera* on soil properties assessed at plot level in invaded and uninvaded areas in coniferous and deciduous forest stands at four occasions.

	Soil moisture[a]	Total soil organic matter[b]	Soil pH[c]
June 2015			
Forest type	$F_{1,4} = 1.79$, $P = 0.252$	$F_{1,4} = 3.72$, $P = 0.126$	$F_{1,4} = 1.97$, $P = 0.233$
Invasion status (presence of *I. glandulifera*)	$F_{1,4} = 10.3$, $\mathbf{P = 0.033}$	$F_{1,5} = 1.20$, $P = 0.324$	$F_{1,5} = 0.43$, $P = 0.540$
Forest type x invasion status	$F_{1,4} = 2.41$, $P = 0.195$	–	–
July 2015			
Forest type	$F_{1,4} = 11.7$, $\mathbf{P = 0.027}$	$F_{1,4} = 7.88$, $\mathbf{P = 0.049}$	$F_{1,4} = 2.31$, $P = 0.203$
Invasion status (presence of *I. glandulifera*)	$F_{1,4} = 23.0$, $\mathbf{P = 0.009}$	$F_{1,4} = 3.48$, $P = 0.136$	$F_{1,4} = 1.76$, $P = 0.255$
Forest type x invasion status	$F_{1,4} = 2.82$, $P = 0.168$	$F_{1,4} = 4.03$, $P = 0.115$	$F_{1,4} = 2.43$, $P = 0.194$
September 2015			
Forest type	$F_{1,4} = 5.33$, $P = 0.082$	$F_{1,4} = 2.48$, $P = 0.190$	$F_{1,4} = 2.08$, $P = 0.223$
Invasion status (presence of *I. glandulifera*)	$F_{1,5} = 25.1$, $\mathbf{P = 0.004}$	$F_{1,5} = 2.48$, $P = 0.176$	$F_{1,5} = 4.83$, $P = 0.080$
Forest type x invasion status	–	–	–
December 2015			
Forest type	$F_{1,4} = 1.82$, $P = 0.249$	$F_{1,4} = 2.34$, $P = 0.201$	$F_{1,4} = 1.47$, $P = 0.293$
Invasion status (presence of *I. glandulifera*)	$F_{1,4} = 9.05$, $\mathbf{P = 0.040}$	$F_{1,5} = 1.11$, $P = 0.341$	$F_{1,5} = 5.50$, $P = 0.066$
Forest type x invasion status	$F_{1,4} = 3.30$, $P = 0.144$	–	–

Significant P values ($P < 0.05$) are printed in bold.
– Excluded from the model after step-wise reduction.
[a] June, July: sqrt(log)-transformed; September: log-transformed.
[b] September: sqrt(log)-transformed.
[c] June, July: sqrt(log)-transformed; September, December: log-transformed.

indicated that plant species composition was differently affected in coniferous and deciduous forests (Table S6).

3.3. Effects of I. glandulifera on richness and composition of the soil fungal community (T-RFLP)

For June samples, T-RFLP analyses revealed a total of 153 operational taxonomic units (OTUs) for the six study sites. In coniferous forests, 115 OTUs were found, 87 (75.7%) in invaded sites and 77 (70.0%) in uninvaded sites. Out of a total of 127 OTUs recorded in deciduous forests, 95 (74.8%) were found in invaded areas and 97 (76.4%) in uninvaded areas (Table S7). Similar numbers were found at the other three sampling occasions (Table S7).

The results of LME analyses showed that the number of OTUs (Fig. 1) differed between invaded and uninvaded areas at all four sampling occasions and in both forest types (Table 2). Tukey post hoc tests showed that the number of OTUs was higher in areas invaded by *I. glandulifera* than in uninvaded areas (June: $P = 0.018$, July: $P = 0.028$, September: $P = 0.005$, December: $P = 0.001$). This effect was more pronounced in coniferous than in deciduous forests (Fig. 1). Furthermore, OTUs' richness increased with increasing soil moisture in June (Spearman rank correlation: $r_s = 0.28$, $n = 62$, $P = 0.030$) and July ($r_s = 0.40$, $n = 62$, $P = 0.001$), and also increased with increasing soil pH in July ($r_s = 0.38$, $n = 62$, $P = 0.002$) and September ($r_s = 0.31$, $n = 61$, $P = 0.014$).

PERMANOVA analyses showed that the presence of *I. glandulifera* caused significant shifts in the soil fungal composition at all four sampling occasions (Table 3). Furthermore, the soil fungal composition also differed between coniferous and deciduous forests in June and December, and was influenced by the amount of leaf litter and by soil pH in June (Table 3). Multiple regression analyses showed that only in July the density of *I. glandulifera* had an effect on soil fungal diversity (Table S8).

3.4. Effects of I. glandulifera on the activity of the soil bacterial community (Ecoplates)

LME analyses showed that the average carbon substrate utilization of soil bacteria (Fig. 2a) was lower in plots invaded by *I. glandulifera* than in uninvaded plots in June 2015 (Tukey post hoc test: $P < 0.001$), both in coniferous and in deciduous forests (Table 4). Carbon substrate utilization was influenced by soil moisture and increased with increasing soil pH (Spearman rank correlation: $r_s = 0.32$, $n = 71$, $P = 0.007$). In December, carbon substrate utilization of soil bacteria did not differ between invaded and uninvaded plots (Fig. 2b), but was influenced by soil moisture (Table 4).

Differences in substrate utilization patterns are a surrogate for changes in the composition of soil bacterial communities. PERMANOVA analyses based on the utilization of the six different substrate classes showed that the presence of *I. glandulifera* caused significant shifts in soil bacterial substrate utilization pattern in June (Table 5). The patterns also differed between coniferous and deciduous forests and were influenced by soil moisture and soil pH (Table 5). In December, the soil bacterial substrate utilization did not differ between invaded and uninvaded plots (Table 5). However, it varied between coniferous and deciduous forests, and was influenced by soil moisture (Table 5). The significant interaction between forest type and invasion status in December indicated that the presence of *I. glandulifera* affected the soil bacterial substrate utilization pattern in coniferous and deciduous forests in different ways (Table 5).

4. Discussion

The present study showed that *I. glandulifera* caused significant shifts in plant species composition, without affecting plant species richness in the above-ground vegetation. As expected, the composition of the soil fungal community differed between invaded and uninvaded plots but, in contrary to our hypothesis, soil fungal communities had a higher OTUs richness in the presence of *I. glandulifera*. Further, contradicting our hypothesis, our study showed that the activity of the soil bacterial community in late spring was lower in plots invaded by *I. glandulifera*, and revealed shifts in substrate utilization patterns of soil bacteria in late spring and late autumn. Our study provides first evidence for different impacts of *I. glandulifera* on deciduous and coniferous forests: the latter seem to respond more sensitively to the plant invasion.

In our study, soil characteristics including soil moisture and soil pH were shown to have an influence on the richness and composition of plant, fungal and bacterial communities. Goldmann et al. (2015, 2016) showed that edaphic factors can influence soil fungal richness and community composition. Other field experiments demonstrated that *I. glandulifera* can effectively change soil characteristics, for example by increasing soil moisture and soil pH in invaded areas (Ruckli et al., 2013, 2014a). Therefore, the influence of soil moisture and soil pH on plant, fungal and bacterial communities can be seen as an indirect effect of the *I. glandulifera* invasion.

Non-native plants increasingly invade forest ecosystems (Nobis, 2008; Zerbe, 2008; Rusterholz et al., 2012; Wagner et al., 2017), and

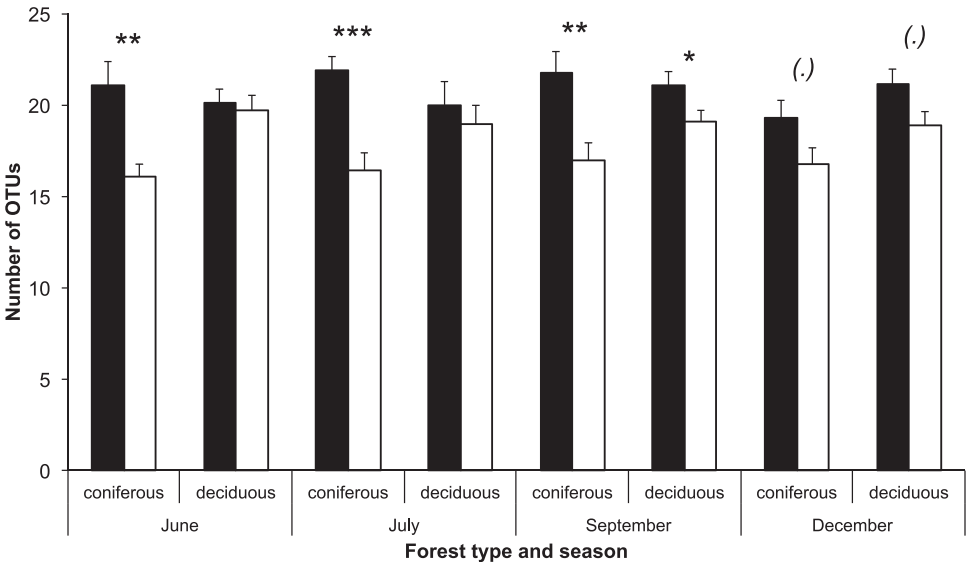

Fig. 1. Number of operational taxonomic units (OTUs) as a measure of fungal diversity in areas invaded by *Impatiens glandulifera* (black bars) and in uninvaded areas (open bars) for the four sampling occasions. Means ± SE per plot; see Table S7 for sample sizes (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, (.) $P < 0.1$; results from unpaired t-tests).

Table 2
Summary of linear mixed-effect model (LME) analyses showing the effects of the presence of *Impatiens glandulifera*, forest type and plot characteristics on soil fungal richness (number of OTUs) assessed at plot level in invaded and uninvaded areas in coniferous and deciduous forest stands.

	June 2015	July 2015	September 2015	December 2015
Forest type	$F_{1,4} = 3.00$, $P = 0.158$	$F_{1,4} = 0.10$, $P = 0.762$	$F_{1,4} = 1.43$, $P = 0.230$	$F_{1,4} = 6.10$, $P = 0.069$
Invasion status (presence of *I. glandulifera*)	$F_{1,4} = 8.04$, $\mathbf{P = 0.047}$	$F_{1,4} = 11.6$, $\mathbf{P = 0.027}$	$F_{1,4} = 16.3$, $\mathbf{P = 0.016}$	$F_{1,4} = 8.30$, $\mathbf{P = 0.035}$
Cover of ground vegetation (%)	–	$F_{1,47} = 1.86$, $P = 0.179$	$F_{1,45} = 3.33$, $P = 0.075$	–
Number of plant species	–	–	$F_{1,45} = 1.45$, $P = 0.234$	$F_{1,51} = 3.89$, $P = 0.054$
Leaf litter (g)[a]	–	–	–	–
Soil moisture (%)	$F_{1,49} = 5.82$, $\mathbf{P = 0.020}$	$F_{1,47} = 7.18$, $\mathbf{P = 0.010}$	$F_{1,45} = 2.32$, $P = 0.135$	–
Soil pH	–	$F_{1,47} = 8.10$, $\mathbf{P = 0.007}$	$F_{1,45} = 7.90$, $\mathbf{P = 0.007}$	$F_{1,51} = 3.65$, $P = 0.062$
Forest type x invasion status	$F_{1,4} = 3.73$, $P = 0.126$	$F_{1,4} = 1.54$, $P = 0.283$	$F_{1,4} = 1.69$, $P = 0.263$	–

Significant P values ($P < 0.05$) are printed in bold.
– Excluded from the model after step-wise reduction.
[a] Log-transformed.

several studies demonstrated how native plant species diversity can be negatively affected by the invasions of alien plants (Hejda et al., 2009; Stoll et al., 2012). In our study, however, plant species richness was not reduced in plots invaded by *I. glandulifera*. The discrepancy between studies could be due to differences in invasion history in the sites investigated. In fact, *I. glandulifera* may not strongly affect native vegetation in the short term (Hejda and Pyšek, 2006; Hulme and Bremner, 2006). However, this invasive species can reduce plant species richness in both the above-ground vegetation and soil seed bank in the long term, with a delay of about 15 years (Rusterholz et al., 2017). Our finding that *I. glandulifera* significantly altered the species composition of native plants is in line with the results of other studies (Hejda and Pyšek, 2006; Tanner et al., 2013; Diekmann et al., 2016).

Mycorrhizal fungi represent the main part of soil fungi in terms of biomass (Nehls, 2008). The "degraded mutualist hypothesis" states that some invasive plants have the potential to inhibit native symbiotic communities leading to an indirectly reduced fitness of native plant species (Vogelsang et al., 2004; Vogelsang and Bever, 2009; Barto et al., 2011). According to this hypothesis, non-mycorrhizal invasive plant species such as *Alliaria petiolata* have the potential to reduce the diversity of native mycorrhizal communities (Stinson et al., 2006; Callaway et al., 2008). Considering *I. glandulifera*, Harley and Harley (1987) reported contradicting results concerning the dependency of *I. glandulifera* on arbuscular mycorrhizal fungi (AMF) (see also Beerling and Perrins, 1993; Clements et al., 2008). More recent studies, however, showed that *I. glandulifera* forms a symbiosis with AMF with a colonization rate of 10–90% in the non-native range (Štajerová et al., 2009; Tanner et al., 2014; Majewska et al., 2015; Gucwa-Przepióra et al., 2016). The observed huge variation in AMF colonization of *I. glandulifera* suggests that this invasive species is not strictly depending on mycorrhizal symbiosis, and that the degree of mycorrhizal colonization is varying according to particular habitat conditions of the sites (de Witte et al., 2017). Interestingly, the higher number of fungal OTUs found in plots invaded by *I. glandulifera* than in uninvaded plots (at all four sampling occasions) contradicted our hypothesis as well as the "degraded mutualist hypothesis". In this context it is important to note that the genetic method applied in our study does not allow to discriminate between the identity and a particular function of the different components of soil fungal communities (e.g. mycorrhizal fungi or decomposers). Therefore, we can only suggest that the observed increase in soil fungal richness may not be a result of an increase in mycorrhizal fungal species, but rather a result of an increase in saprophytic fungi, another important component of the soil fungal community responsible for the decomposition of dead organic matter. In our study, leaf litter biomass in invaded plots was 37% lower than in uninvaded ones (data not shown). This indicates a higher activity of decomposers in association with a higher saprophytic fungal diversity. The recorded shifts in the soil fungal composition between invaded and uninvaded areas at all four sampling occasions could also reflect a change in the proportion of mycorrhizal to saprophytic fungi. The observed shift in fungal composition can be explained by a decreased number of mycorrhizal plant species and an increased number of non-mycorrhizal species in invaded areas (Fig. S1). Furthermore, the lower number of plant species depending on EMF symbioses found in invaded compared to uninvaded areas in our study (Fig. S2), together with the fact that ectomycorrhizal fungi (EMF) tend to be more host-specific than AMF (Borowicz and Juliano, 1991) suggest that effects of *I. glandulifera* are more pronounced in EMF than in AMF. In fact, Tanner and Gange (2013) presumed that *I. glandulifera* does not completely eliminate mycorrhizal fungi from invaded stands, but may eliminate some mycorrhizal fungal species which are more beneficial to native plants.

The arrival of a new, invasive plant species can lead not only to changes in the soil fungal community, but can also affect the structure and activity of soil bacterial communities, which play an important role in the functioning of natural ecosystems (Kourtev et al., 2002). Interestingly, we found a lower bacterial activity in invaded areas than in uninvaded areas in June but not in December, contradicting our hypothesis. This result is also in contrast with the expectation of a higher

Table 3
Summary of PERMANOVA analyses testing the effects of forest type, presence of *Impatiens glandulifera* and plot characteristics on soil fungal composition (presence/absence of OTU) assessed at plot level at four occasions.

	June 2015	July 2015	September 2015	December 2015
Forest type	$F_{1,53} = 6.67$, $\mathbf{P = 0.001}$	$F_{1,52} = 6.88$, $P = 0.106$	$F_{1,52} = 6.35$, $P = 0.130$	$F_{1,57} = 5.02$, $\mathbf{P = 0.001}$
Invasion status (presence of *I. glandulifera*)	$F_{1,53} = 2.53$, $\mathbf{P = 0.001}$	$F_{1,52} = 1.77$, $\mathbf{P = 0.014}$	$F_{1,52} = 1.52$, $\mathbf{P = 0.040}$	$F_{1,57} = 1.76$, $\mathbf{P = 0.028}$
Cover of ground vegetation (%)	$F_{1,53} = 1.64$, $P = 0.092$	$F_{1,52} = 1.41$, $P = 0.242$	$F_{1,52} = 1.85$, $P = 0.103$	$F_{1,57} = 2.18$, $P = 0.069$
Number of plant species	$F_{1,53} = 1.90$, $P = 0.704$	$F_{1,52} = 1.85$, $P = 0.207$	$F_{1,52} = 1.51$, $P = 0.515$	$F_{1,57} = 1.84$, $P = 0.120$
Leaf litter (g)[a]	$F_{1,53} = 1.74$, $\mathbf{P = 0.036}$	$F_{1,52} = 1.09$, $P = 0.468$	$F_{1,52} = 1.01$, $P = 0.431$	$F_{1,57} = 1.28$, $P = 0.257$
Soil moisture (%)	$F_{1,53} = 1.62$, $P = 0.102$	$F_{1,52} = 1.34$, $P = 0.289$	$F_{1,52} = 1.13$, $P = 0.613$	–
Soil pH	$F_{1,53} = 2.38$, $\mathbf{P = 0.010}$	$F_{1,52} = 1.09$, $P = 0.822$	$F_{1,52} = 1.34$, $P = 0.500$	$F_{1,57} = 1.85$, $P = 0.095$
Forest type x invasion status	$F_{1,53} = 1.44$, $P = 0.107$	$F_{1,52} = 1.09$, $P = 0.397$	$F_{1,52} = 1.36$, $P = 0.158$	$F_{1,57} = 1.88$, $\mathbf{P = 0.016}$

Significant P values ($P < 0.05$) are printed in bold.
– Excluded from the model after step-wise reduction.
[a] Log-transformed.

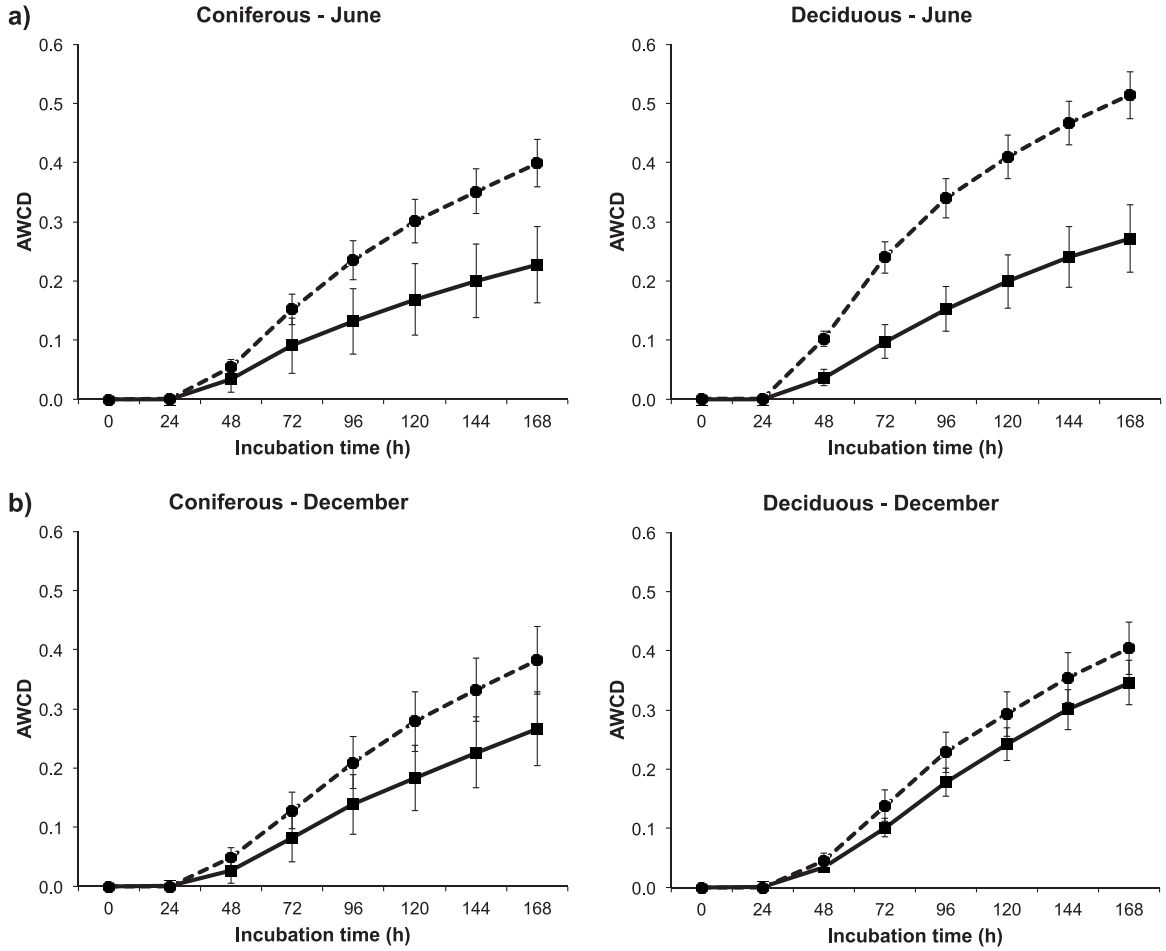

Fig. 2. Average well colour development (AWCD; OD 590 nm) of carbon substrate utilization pattern of soil bacteria using Biolog Ecoplates in areas invaded by *Impatiens glandulifera* (solid lines) and in uninvaded areas (dashed lines) in both coniferous and deciduous forest stands. Means ± SE are shown (n = 6 plots per area, 3 areas per forest type). (a) June 2015, (b) December 2015.

Table 4
Summary of linear mixed-effect model (LME) analyses showing the effects of forest type, presence of *Impatiens glandulifera* and soil characteristics on average carbon substrate utilization after 168 h incubation in both invaded and uninvaded areas in coniferous and deciduous forest stands.

	June 2015	December 2015
Forest type	$F_{1,4} = 1.11$, $P = 0.351$	$F_{1,4} = 1.29$, $P = 0.319$
Invasion status (presence of *I. glandulifera*)	$F_{1,5} = 7.95$, $\mathbf{P = 0.037}$	$F_{1,4} = 3.73$, $P = 0.126$
Soil moisture (%)	$F_{1,57} = 18.6$, $\mathbf{P < 0.001}$	$F_{1,58} = 16.3$, $\mathbf{P < 0.001}$
Soil pH	$F_{1,57} = 15.5$, $\mathbf{P < 0.001}$	$F_{1,58} = 2.07$, $P = 0.155$
Forest type x invasion status	–	$F_{1,4} = 5.66$, $P = 0.076$

Significant *P* values ($P < 0.05$) are printed in bold.
– Excluded from the model after step-wise reduction.

bacterial activity with increasing leaf litter decomposition in invaded plots. Pattison et al. (2016) found a modified soil bacterial community structure in areas invaded by *I. glandulifera,* with a significant increase in bacterial biomass. The decreased bacterial activity recorded in invaded areas in our study could be explained by a possible negative influence of the naphthoquinones on soil bacteria, since plant allelopathic compounds often affect soil microbial communities, including soil bacteria (Cipollini et al., 2012).

Our study provides some evidence that *I. glandulifera* differently affect deciduous and coniferous forests: coniferous forests seem to be more sensitive against *I. glandulifera* invasion. A similar pattern was observed by Lorenzo et al. (2013) in forests invaded by *Acacia dealbata*. The differences in fungal OTUs richness between invaded and uninvaded areas, as well as in the number of mycorrhizal vs. non-mycorrhizal plant species, are all more pronounced in coniferous forests than in deciduous forests. In fact, the coniferous forest stands examined in our study were dominated by EMF-dependent trees (90% of all trees; L. Gaggini, unpublished data). Previous field experiments on the impact of *I. glandulifera* revealed more pronounced reductions in degree of mycorrization in EMF-dependent *Fagus sylvatica* (–60%; Ruckli et al., 2016), than in AMF-dependent *Acer pseudoplatanus* (–40%; Ruckli et al., 2014a).

Table 5
Summary of PERMANOVA analyses testing the effects of forest type, presence of *Impatiens glandulifera* and plot characteristics on carbon substrate utilization pattern of six different substrate classes after 168 h incubation.

	June 2015	December 2015
Forest type	$F_{1,66}$ = 1.26, ***P* = 0.001**	$F_{1,65}$ = 3.02, ***P* = 0.009**
Invasion status (presence of *I. glandulifera*)	$F_{1,66}$ = 11.4, ***P* = 0.001**	$F_{1,65}$ = 2.18, P = 0.065
Leaf litter (g)[a]	–	$F_{1,65}$ = 1.21, P = 0.663
Soil moisture (%)	$F_{1,66}$ = 3.77, ***P* = 0.006**	$F_{1,65}$ = 4.83, ***P* = 0.003**
Soil pH	$F_{1,66}$ = 4.05, ***P* = 0.005**	$F_{1,65}$ = 2.17, P = 0.123
Forest type x invasion status	–	$F_{1,65}$ = 3.22, ***P* = 0.030**

Significant P values ($P < 0.05$) are printed in bold.
– Excluded from the model after step-wise reduction.
[a] Log-transformed.

5. Conclusions

Our study showed that the invasion of the annual plant *I. glandulifera* can affect and alter soil fungal and bacterial communities in deciduous and coniferous forests. This may have consequences on ecosystem processes such as decomposition and nutrient cycling. We argue that the detected changes in soil fungal and bacterial communities may be an indirect result of the alterations in soil physical and chemical properties induced by the invasion of *I. glandulifera*, in combination with the release of naphthoquinones into the soil.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.apsoil.2017.11.021

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Supplementary material Chapter II

Table S1

Characteristics of the forest areas invaded by *Impatiens glandulifera* and of uninvaded areas located in coniferous and deciduous forest stands near Brislach (Basel-Landschaft), Switzerland

	Coniferous forests						Deciduous forests					
	AREA 1		AREA 2		AREA 3		AREA 4		AREA 5		AREA 6	
	Invaded	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded
Number of shrubs	5	125	84	41	19	47	16	23	28	90	28	17
Number of shrub species	2	8	6	6	4	8	4	6	5	7	6	6
Shrub height (cm, mean)	164	130	138	126	158	109	154	119	137	121	195	199
Number of tree stems	9	25	7	11	3	24	3	14	17	3	7	23
Number of tree species	2	4	4	5	1	5	2	5	6	2	4	5
Tree diameter (cm, median)	28.6	11.8	41.4	27.7	31.2	23.6	31.8	29.3	8.6	63.7	44.6	20.1
Diameter at breast height (cm, mean)	30.5	25.3	48.4	40.8	31.4	24.9	34.0	26.4	9.6	54.6	39.0	24.2
Canopy closure (%)	43	87	85	89	49	79	55	86	57	56	74	89
Cover of aboveground vegetation (%)	97	90	83	88	100	92	88	55	94	89	86	39
Cover of *Impatiens glandulifera* (%)	67	0	40	0	51	0	78	0	28	1	33	0
Cover of *Rubus* sp. (%)	14	21	21	15	48	42	44	39	3	42	1	0
Cover of leaf litter (%)	9	10	26	10	10	15	60	79	5	10	8	47

Table S2

Cover, biomass, density and height of *Impatiens glandulifera* in the invaded areas at three sampling occasions (mean ± SE, n = 6 plots per area). In December, all *I. glandulifera* plants were already decaying (no measurements available). Areas 1–3 were located in coniferous forests, areas 4–6 in deciduous forests

		Cover of *I. glandulifera* (%)	Biomass of *I. glandulifera* (g m^{-2})	Density of *I. glandulifera* (m^{-2})	Height of *I. glandulifera* (cm)
June 2015	Area 1	75 ± 6	303 ± 53	1954 ± 431	35.8 ± 2.6
	Area 2	63 ± 4	385 ± 70	1000 ± 172	49.3 ± 2.1
	Area 3	70 ± 6	267 ± 47	2208 ± 424	34.8 ± 1.2
	Area 4	93 ± 2	548 ± 48	3883 ± 513	38.0 ± 3.0
	Area 5	73 ± 3	291 ± 52	1646 ± 372	40.9 ± 2.3
	Area 6	68 ± 5	319 ± 54	1154 ± 193	41.1 ± 2.6
July 2015	Area 1	76 ± 1	1023 ± 169	1058 ± 207	100.1 ± 6.9
	Area 2	53 ± 2	533 ± 43	479 ± 107	91.6 ± 2.9
	Area 3	75 ± 4	735 ± 138	638 ± 89	90.1 ± 5.1
	Area 4	55 ± 6	889 ± 348	946 ± 211	77.5 ± 11.0
	Area 5	63 ± 6	600 ± 75	600 ± 54	90.5 ± 4.1
	Area 6	72 ± 8	840 ± 134	617 ± 94	97.6 ± 9.9
September 2015	Area 1	72 ± 5	970 ± 71	350 ± 49	122.3 ± 7.1
	Area 2	38 ± 4	420 ± 61	221 ± 28	93.8 ± 3.5
	Area 3	73 ± 4	951 ± 94	271 ± 41	109.0 ± 8.5
	Area 4	57 ± 13	663 ± 244	338 ± 84	87.2 ± 14.3
	Area 5	47 ± 2	406 ± 54	350 ± 37	92.8 ± 4.9
	Area 6	44 ± 5	674 ± 147	175 ± 18	107.1 ± 12.5

Table S3

Summary of soil characteristics recorded at plot level. Mean values ± SE, *n* = 6 plots per area

	Coniferous forests						Deciduous forests					
	AREA 1		AREA 2		AREA 3		AREA 4		AREA 5		AREA 6	
	Invaded	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded	Invaded	Uninvaded
June 2015												
Soil moisture (%)	47.7 ± 5.2	27.9 ± 3.0	32.9 ± 1.3	31.2 ± 1.8	39.3 ± 3.1	22.2 ± 1.2	31.9 ± 1.4	25.5 ± 1.1	30.2 ± 0.8	24.8 ± 1.4	30.2 ± 0.8	31.3 ± 1.2
Total soil organic matter (%)	20.0 ± 5.4	11.2 ± 0.5	10.8 ± 1.0	12.6 ± 1.8	13.9 ± 2.4	11.5 ± 1.0	13.5 ± 1.3	9.0 ± 0.4	8.1 ± 0.3	10.3 ± 0.3	9.8 ± 0.2	10.9 ± 0.8
Soil pH	4.6 ± 0.2	4.8 ± 0.1	4.5 ± 0.2	4.6 ± 0.1	4.6 ± 0.1	4.3 ± 0.0	4.6 ± 0.2	4.5 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	5.3 ± 0.1	5.7 ± 0.1
July 2015												
Soil moisture (%)	36.5 ± 2.3	18.3 ± 1.2	25.4 ± 1.3	18.9 ± 1.3	33.9 ± 2.3	13.3 ± 0.3	18.7 ± 1.4	12.6 ± 0.5	19.3 ± 0.9	12.8 ± 1.0	18.7 ± 0.9	17.6 ± 0.8
Total soil organic matter (%)	18.4 ± 3.6	10.9 ± 0.7	11.5 ± 0.9	11.8 ± 1.6	19.6 ± 3.4	10.1 ± 0.3	11.3 ± 0.7	8.3 ± 0.4	7.5 ± 0.2	10.3 ± 0.4	9.9 ± 0.4	10.8 ± 0.4
Soil pH	4.5 ± 0.1	4.6 ± 0.1	4.3 ± 0.1	4.5 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.6 ± 0.1	4.6 ± 0.0	4.9 ± 0.1	5.2 ± 0.1	5.5 ± 0.1
September 2015												
Soil moisture (%)	42.7 ± 4.7	17.7 ± 1.3	25.2 ± 1.0	17.5 ± 0.8	31.1 ± 1.6	14.7 ± 0.6	24.4 ± 2.0	10.2 ± 0.3	21.6 ± 0.9	14.3 ± 0.7	22.0 ± 1.3	18.5 ± 1.2
Total soil organic matter (%)	20.4 ± 5.2	11.1 ± 0.3	11.8 ± 1.1	11.8 ± 1.5	12.5 ± 1.6	10.2 ± 0.4	13.8 ± 0.8	7.8 ± 0.4	8.3 ± 0.3	9.9 ± 1.2	11.3 ± 0.4	11.6 ± 0.6
Soil pH	4.5 ± 0.1	4.9 ± 0.1	4.3 ± 0.2	4.5 ± 0.2	4.4 ± 0.0	4.3 ± 0.1	4.4 ± 0.1	4.5 ± 0.0	4.6 ± 0.0	5.0 ± 0.1	5.3 ± 0.1	5.5 ± 0.1
December 2015												
Soil moisture (%)	44.5 ± 4.9	24.3 ± 1.4	28.1 ± 1.1	26.5 ± 0.7	37.6 ± 3.0	21.7 ± 0.3	29.6 ± 1.0	22.3 ± 0.4	27.0 ± 0.8	26.8 ± 0.7	28.8 ± 0.5	27.0 ± 0.7
Total soil organic matter (%)	19.9 ± 5.0	10.8 ± 0.4	8.9 ± 0.5	12.4 ± 1.5	13.7 ± 2.2	9.5 ± 0.5	12.4 ± 0.8	7.8 ± 0.3	7.7 ± 0.3	10.4 ± 0.7	10.1 ± 0.4	10.4 ± 0.5
Soil pH	4.5 ± 0.1	4.8 ± 0.1	4.2 ± 0.1	4.5 ± 0.2	4.5 ± 0.1	4.3 ± 0.0	4.3 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	5.0 ± 0.1	5.33 ± 0.1	5.6 ± 0.1

Table S4

List of plant species recorded in the ground vegetation at the plot level in areas invaded by *Impatiens glandulifera* and in uninvaded areas located in coniferous and deciduous forest stands near Brislach (Basel-Landschaft), Switzerland

	Coniferous forests		Deciduous forests	
Species	Invaded	Uninvaded	Invaded	Uninvaded
Abies alba Mill.	x	x	x	
Acer campestre L.	x			
Acer pseudoplatanus L:	x	x	x	x
Agrostis capillaris L.	x	x	x	x
Agrostis gigantea Roth	x	x		
Anemone nemorosa L.	x	x	x	x
Arum maculatum L.		x	x	x
Athyrium filix-femina (L.) Roth	x	x	x	x
Brachypodium sylvaticum (Huds.) P. Beauv.	x	x		
Cardamine flexuosa With.	x	x	x	
Carex pendula Huds.	x		x	
Carex remota L.	x	x		
Carex sylvatica Huds.	x	x	x	x
Carpinus betulus L.	x	x	x	x
Circaea lutetiana L.	x	x	x	x
Dryopteris dilatata (Hoffm.) A. Gray	x	x	x	x
Dryopteris filix-mas (L.) Schott			x	
Epilobium tetragonum L. s.l.			x	
Fagus sylvatica L.		x	x	x
Festuca altissima All.	x			x
Festuca gigantea (L.) Vill.	x			
Fragaria vesca L.		x	x	
Fraxinus excelsior L.	x	x	x	x
Galeopsis tetrahit L.	x	x	x	x
Galium aparine L.	x			x
Galium elongatum C. Presl	x			
Galium odoratum (L.) Scop.	x	x		x
Geranium robertianum L. s.l.	x			x
Geum urbanum L.	x		x	x
Glechoma hederacea L. s.l.	x	x		x
Hedera helix L.	x	x	x	x
Impatiens glandulifera Royle	x	x	x	x
Impatiens noli-tangere L.	x			
Juncus effusus L.	x	x	x	x
Lamium galeobdolon (L.) L. s.l.	x	x	x	x
Lapsana communis L. s.l.		x	x	x
Lotus pedunculatus Cav.	x			
Luzula pilosa (L.) Willd.	x	x	x	x
Lysimachia nemorum L.	x	x	x	x
Mercurialis perennis L.		x	x	x
Moehringia trinervia (L.) Clairv.	x	x	x	x

	Coniferous forests		Deciduous forests	
Species	Invaded	Uninvaded	Invaded	Uninvaded
Oxalis acetosella L.	x	x	x	
Paris quadrifolia L.	x	x		x
Phegopteris connectilis (Michx.) Watt	x			
Picea abies (L.) H. Karst.		x		x
Plantago major L. s.l.				x
Primula elatior L. s.l.			x	
Prunella vulgaris L.	x	x		x
Prunus avium L.		x	x	x
Quercus robur L.		x		x
Quercus rubra L.		x		x
Ranunculus ficaria L.	x	x	x	
Ranunculus repens L.	x	x	x	
Rubus sp.	x	x	x	x
Rumex conglomeratus Murray	x			
Rumex obtusifolius L.	x			
Sambucus nigra L.	x	x	x	x
Sanicula europaea L.		x		
Stachys sylvatica L.	x	x	x	x
Taraxacum officinale aggr.				x
Urtica dioica L.			x	x
Veronica montana L.	x	x	x	x
Veronica officinalis L.				x
Viburnum opulus L.		x		
Viola reichenbachiana Boreau	x	x		

Table S5

Summary of linear mixed-effect model (LME) analyses showing the effects of the presence of *Impatiens glandulifera*, forest type and plot characteristics on plant species richness (number of plant species) and on plant species diversity (Shannon index) assessed at plot level in invaded and uninvaded areas (each n = 6, with 6 plots per area) in both coniferous and deciduous forests

	Number of plant species	Shannon index
Forest type	$F_{1,4} = 2.26$, $P = 0.207$	$F_{1,4} = 2.46$, $P = 0.192$
Invasion status (presence of *I. glandulifera*)	$F_{1,4} = 0.04$, $P = 0.854$	$F_{1,4} = 0.59$, $P = 0.485$
Cover of ground vegetation (%)	–	$F_{1,58} = 2.65$, $P = 0.109$
Soil moisture (%, mean)	$F_{1,58} = 1.14$, $P = 0.291$	–
Soil pH (mean)	$F_{1,58} = 11.1$, **$P = 0.002$**	$F_{1,58} = 7.61$, **$P = 0.008$**
Forest type x invasion status	$F_{1,4} = 2.89$, $P = 0.164$	$F_{1,4} = 1.79$, $P = 0.252$

Significant P values ($P < 0.05$) are printed in bold

– Excluded from the model after step-wise reduction

Table S6

Summary of PERMANOVA testing the effects of forest type, presence of *Impatiens glandulifera* and plot characteristics on plant species composition in the ground vegetation. Cover values of *I. glandulifera* were not considered in this analysis

Forest type	$F_{1,65} = 5.85$, $\mathbf{P = 0.001}$
Invasion status (presence of *I. glandulifera*)	$F_{1,65} = 4.77$, $\mathbf{P = 0.001}$
Leaf litter (g) [a]	$F_{1,65} = 2.33$, $P = 0.200$
Soil moisture (%, mean)	$F_{1,65} = 3.63$, $\mathbf{P = 0.001}$
Soil pH (mean)	$F_{1,65} = 6.56$, $P = 0.353$
Forest type x invasion status	$F_{1,65} = 1.75$, $\mathbf{P = 0.010}$

Significant P values ($P < 0.05$) are printed in bold

[a] Log-transformed

Table S7

List of operational taxonomic units (OTUs) recorded at the plot level in areas invaded by *Impatiens glandulifera* and in uninvaded areas in coniferous and deciduous forest stands near Brislach (Basel-Landschaft), Switzerland. OTUs are listed with their frequencies of occurrence, and serve as a measure for soil fungal diversity. (**a**) June, (**b**) July, (**c**) September, (**d**) December 2015

(a)

JUNE 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 13)	Uninvaded (n = 15)	Invaded (n = 16)	Uninvaded (n = 18)
50.5	4	2	4	2
56.9				1
62.3		1		
65.7	13	15	16	18
71.8	2			3
73.3	2	7	2	5
74.3	3	1	2	
77.3		1		
78.9	1			
82.1				1
83.6	6	3	9	8
85.2	1		1	
91.7			1	
93.4			3	
95.3		5	2	6
96.0	6	2	4	7
96.8	1	1		
97.8				2
101.2	6	5	11	6
102.5		1		3
107.5	1			2
111.7		1		
112.7	1			
114.5	1		1	
116.7			1	
119.0			1	
123.2	8	13	12	16
127.1	2			
129.5			1	
135.6	5	1	2	2
139.0	3		3	
142.8	1			
143.8	1		1	
149.1	1			
152.2				1
153.8				1
155.2	1			2
157.3			9	
162.0	1		7	6
163.2			1	1
164.7	1			

JUNE 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 13)	Uninvaded (n = 15)	Invaded (n = 16)	Uninvaded (n = 18)
167.5			2	1
168.5	2	1		
170.1	1	1		3
171.6				1
173.2				1
175.3			2	
187.7		1	1	
189.2	1			
190.4	1	1		
191.6		1	1	2
193.1	9	3	6	1
195.1		6	1	3
196.0		2	1	1
197.0	1			
198.1		1		
199.6		2	1	1
201.0				2
201.9	1		2	2
203.3	2		1	
204.4	1			
206.2	5		1	
207.1	13	14	16	16
208.3	6		3	9
210.4				2
211.7			1	1
213.0	3	1	2	
216.2	1	1	1	2
218.7	6	1		1
220.8	1		1	
223.6			2	1
225.3				1
227.6		1	1	2
229.5				1
231.3	1	1	1	1
233.2	4	3	5	6
234.5	9	6	9	6
235.3	2	2	4	6
236.3	3	2	3	2
237.4			2	3
239.0	1			1
240.0	4	7	1	3
240.9	9		15	1
242.3		1		3
244.1		3	1	2
245.6	1		1	
248.5	3		1	2
249.8	3		3	2
251.1	2		3	4
252.4	11	8	2	7
253.3	3	3	10	10
254.6	4		2	3
255.5	2	3	3	

JUNE 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 13)	Uninvaded (n = 15)	Invaded (n = 16)	Uninvaded (n = 18)
256.7				4
257.9	1		3	8
258.8	11	12	14	15
259.9	6	3	9	2
261.4	4	4	10	6
262.7	2		1	1
263.9	1	4	3	3
264.9	13	13	14	18
265.9	6	4	10	8
266.8	1	1	3	1
269.1	3	3	2	4
269.9		1	2	11
271.7	3	1	1	2
272.6	1	7	5	4
273.8		3	2	
275.3			1	5
277.5	1			1
279.3				1
281.6		1	2	1
283.8		2		
285.0	2	1	1	1
286.1		1		
287.8		1	2	1
289.0	2	2	1	
290.2	2		2	2
291.6		2		2
292.4		1	1	4
293.8	1	2	1	2
295.0		3	1	
295.9			2	1
297.8	1	1	1	5
298.7	1	8	5	6
300.2	2	8	2	7
301.2	4	1		1
302.2		3		
304.0	1	3		1
305.0	1			
309.7		1		
312.2	4	3	3	
313.6			3	4
319.3			2	
326.8			1	
328.0		1		
330.8	1	1		
331.9		1	1	2
336.0			1	
337.5				3
339.2		1		
341.2	3	1	1	
343.9	1			1
346.0				1
347.6				4

JUNE 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 13)	Uninvaded (n = 15)	Invaded (n = 16)	Uninvaded (n = 18)
349.0				1
350.6			1	
356.8	1		1	
413.2				1
424.6	4	1	1	
435.2	1			
458.6				1
478.5	1			
TOTAL	87	77	95	97

(b)

JULY 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 15)	Uninvaded (n = 15)	Invaded (n = 15)	Uninvaded (n = 17)
50.6	1	4		2
53.1				1
57.1	1			
58.9		1		
62.0	1			1
63.7	1			
65.8	15	15	15	17
72.6	4	1	2	2
73.7		7	1	6
74.3	4	3	1	
78.8	1	1		1
82.0				1
83.5	6	1	8	7
84.1	1		2	3
94.3	2	1		
95.3	5	5	5	10
96.3	1	2		1
97.9		1	1	2
101.1	7	3	10	4
102.4		1		1
104.5				1
107.2	1			
116.5				1
119.3	1		1	
123.2	8	9	10	15
125.8				2
126.8	2			2
129.7			2	1
132.5	2			2
135.5	3		1	
136.2		3		
137.6				1
138.6	1		1	
139.6		1		
143.8		2		
147.2				1
148.2				1
148.9	2			

JULY 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 15)	Uninvaded (n = 15)	Invaded (n = 15)	Uninvaded (n = 17)
150.4	2			
152.1				1
154.8	3			
157.2			5	1
159.1	2			1
160.3	2			
161.2	2			
162.0	7		8	5
163.9	1			
168.6	3			
170.2	1			1
171.4				1
175.6			2	
184.9		1		
188.9	1	1		
190.3		2	1	
191.5		2		
193.1	6	3	7	
194.3	1	2		3
195.4		4	1	
197.3				1
199.0		1		
200.8		1		2
202.0	2		2	4
203.2	1	1	1	2
204.9		2		
205.9			1	1
207.2	15	13	15	16
208.2	4	1	8	4
210.2		1	1	3
212.0		1		2
213.0	1		1	
215.4			1	
216.6		1	1	
218.0	4			1
218.8	5	2		2
226.3		1		1
227.2			2	
227.9	1			1
230.1	2	1		
232.1	2			1
233.5	6	2	4	2
234.6	7	6	7	8
235.3	4	1	4	6
236.4	2	4	3	
237.5	3		1	6
240.0	3	6	2	4
240.9	13	1	13	
242.2				1
243.6				1
245.6	2			
247.5	1			

JULY 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 15)	Uninvaded (n = 15)	Invaded (n = 15)	Uninvaded (n = 17)
248.5	3		4	1
249.6	10	1	6	2
251.2	3	1	3	3
252.5	9	8	4	7
253.4	6	2	6	11
254.5	3	1	3	4
255.3	2	1	3	1
256.8			2	4
258.0	4	6	5	11
258.9	12	8	12	13
260.0	9	3	11	
261.4	6	4	5	7
262.5	1		5	1
263.5	6	4	2	5
264.6	9	9	11	15
265.6	9	6	10	4
266.8	1	1	1	2
269.0	3	4	1	4
269.7	2	2	2	5
270.7	5	1	1	
272.3	2	7	2	7
273.1		3	1	2
274.6		1	1	3
275.7			2	2
278.2			1	
280.4	1	1		
281.6	3		5	
284.2		2		
285.6	1			
287.6		2		1
288.8	2			
290.9		5	2	1
292.7	1	1		3
293.9	1	3	1	
294.9	1	3		
295.8			1	
297.8	3	3	1	7
298.6		6	3	2
300.1	2	8	1	8
301.1	5	1	1	
302.7		1		
304.0		3	1	1
305.7	2	1		
306.5				1
310.3		4	1	
312.0	5	3	5	
313.5			6	4
315.2	3			
326.7				1
330.2			1	
331.5			2	
337.9			3	1

JULY 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 15)	Uninvaded (n = 15)	Invaded (n = 15)	Uninvaded (n = 17)
340.9	3		1	1
341.6	1	1	1	
342.5			1	1
343.3			1	1
344.7	1			
347.2			1	3
348.6				1
350.8		1		
355.1		1		
356.8			1	
361.5				1
385.2	3			
410.7				1
417.9		1		1
424.7	3		1	
435.3	3			
458.8			4	
TOTAL	92	83	85	94

(c)

SEPTEMBER 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 12)	Uninvaded (n = 16)	Invaded (n = 15)	Uninvaded (n = 18)
50.8	5	4	4	1
51.7		2	1	1
58.1		1		
65.6	12	16	15	18
67.0			1	
70.9			1	
72.7	3	2	1	2
73.8	3	7	2	6
77.4		2		
79.0	1	1		
82.1		1		3
83.8	3	1	7	10
85.7	1			
90.6	1			
95.1	2	5	1	2
96.1	4	4	9	7
97.4				2
99.0	1			
101.1	7	5	8	5
102.2		1	1	2
108.2			1	1
114.6	1			
121.9				1
123.3	3	10	11	17
127.1	1			
128.4	1			
129.5			1	
133.0	1	1		1
136.0	2	4	3	1

SEPTEMBER 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 12)	Uninvaded (n = 16)	Invaded (n = 15)	Uninvaded (n = 18)
138.4	2		2	3
143.1		1		1
149.2			1	1
151.6	1			
155.2	3			
156.2	1			
157.4	1		8	1
159.0			1	
160.9	1			
162.1	4	1	11	5
164.6	1		1	
168.4			1	
170.0	1	1	1	1
171.3	1			1
175.6	2			
190.4	2	2		
191.4		2	2	1
192.6	1	1	1	
193.5	5	1	4	1
194.9	1	4	1	
195.9		1		
197.0		2		1
199.1		1		
201.6			2	2
203.1	1		3	1
204.2	1			
205.3		1		1
207.1	12	15	15	17
208.2	5		5	7
210.6	1	2		3
212.6	1		1	1
216.1	2	1	5	1
218.6	6	2		1
224.0	1			
225.2	1			
226.5	2	1		2
227.8	2		1	
230.5	1	1	2	1
233.1	4	4	4	3
234.2	10	4	4	5
235.2	2	5	4	8
236.3	1	2	4	
237.2			5	5
239.2	2	5	2	6
240.8	9	5	14	3
242.5	1	1	2	5
245.5	3		1	
247.0		1		
248.7	1	1		2
249.5	3	1	3	1
251.3	3	1	2	5
252.4	8	7		9

SEPTEMBER 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 12)	Uninvaded (n = 16)	Invaded (n = 15)	Uninvaded (n = 18)
253.4	3	1	10	10
254.5	3	3		5
255.5	2	1	1	1
256.4		2	3	4
257.9	4	6	6	9
258.8	7	11	15	13
260.0	8	1	11	5
261.3	4	3	5	8
262.5	2		7	3
263.4	4	4	1	2
264.7	8	9	11	15
265.6	7	7	11	5
266.5	1	3	1	3
267.3	2	1	2	
269.0	2	4	3	9
269.8	1	3	1	5
271.9	4	4	2	1
272.6	1	3	1	6
273.9	1	3	1	
275.5	1	1	4	4
277.0		1		
280.6	2			
281.7		1	3	2
284.7	2	1	1	
287.3	1	2		1
288.5	2	3		1
290.8	1	1	1	3
292.1		2	1	4
292.7		2		1
294.0	2	3	1	3
296.0			4	
298.3	1	8	5	8
300.2	1	10	3	6
301.3	3	3	1	
302.3		2		
303.3				2
304.2	1	2		2
305.9				1
310.3		2		1
311.1		3	1	
312.2	3	1	2	
313.8		1	5	2
315.1	2	1		
319.1			1	
322.2				1
329.2			1	
330.3			1	
331.6	1		2	4
334.2		1	1	
337.8			1	1
340.3		1		
341.4	2		1	1

SEPTEMBER 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 12)	Uninvaded (n = 16)	Invaded (n = 15)	Uninvaded (n = 18)
342.6	1			
347.2		1		3
350.3		1		
357.5		1		
360.4	1			
361.4				1
397.1		1		
417.7	1	2		2
422.1	1			
424.5	2		2	
430.7	1			
434.9	2			
445.5	1			
458.9			2	
TOTAL	99	92	87	88

(d)

DECEMBER 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 15)	Uninvaded (n = 16)	Invaded (n = 17)	Uninvaded (n = 17)
50.4	4	5	5	7
51.5		1		1
53.0			1	
57.7			2	1
62.7	1			1
65.5	15	16	17	17
70.6	1		1	1
72.0	2			
72.8		6	1	1
73.8	3	5	4	6
75.3				1
79.0	2			
82.1				1
83.6	2	2	5	5
84.1	3		1	4
85.6	3		1	1
88.6			1	
93.5	1		1	
95.0		2	7	2
95.7	11	11	5	4
97.6	1	1	3	3
101.1	7	6	13	5
102.4		3	1	2
108.2	1			
113.6	1			1
114.6	1			
116.5			1	
119.0			1	
121.0			1	
123.1	9	12	14	15
126.6	3			1
127.6	1			

DECEMBER 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 15)	Uninvaded (n = 16)	Invaded (n = 17)	Uninvaded (n = 17)
129.5			1	
135.6	2	2	2	
138.6	3	1	3	
142.6	2	1		
143.8	1			1
146.5				1
151.3				1
153.6			2	
155.9	1			
157.4	1		6	1
159.2			1	
161.8	1		7	
162.3		1	2	5
164.8	1		3	
168.2	2			
170.0	1			2
171.4				1
172.5	1			
174.6	1		1	
175.7	2			
179.7	1			
190.0	4		1	
190.5		1	1	
191.6		1		1
193.3	6	3	6	
195.1	2	1	3	
196.1		1	1	1
199.1		1		
201.2			1	1
202.0			1	2
203.6	1		1	1
204.9	1			1
207.4	15	15	17	17
208.2	5	1	5	6
210.4		1	2	2
212.2	1			2
214.2			1	
215.9		1	4	2
218.5	7	5	1	3
221.9	1	1		1
224.1				1
226.9	1			
227.9			1	
230.1		1	2	
232.2		1	1	
233.2	3	3		1
234.4	10	6	8	5
235.3		1	3	2
236.4	4	5	7	5
238.1	1			
239.5	2	7	4	6
240.8	9	4	11	1

DECEMBER 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 15)	Uninvaded (n = 16)	Invaded (n = 17)	Uninvaded (n = 17)
243.2		1	1	2
245.5	1			
247.5		1	1	
248.3	2			3
249.7	1		3	2
250.8	2		3	5
252.3	10	9	4	10
253.2	1	4	7	9
254.6	2	1	4	5
255.8	2	2	2	6
257.5	1	2	11	9
258.4	12	14	11	13
259.4	11	2	7	3
260.3	3	2	4	2
261.3		2	5	4
262.3	4		8	
263.7	5	7	8	6
264.9	14	13	12	14
265.9	7	1	9	2
267.0		1	1	3
268.2			2	
269.0	3	2	2	8
271.0	2	2		1
271.9	4	3	4	7
272.6	1	4		
274.1		4	1	1
275.1			1	2
277.1	1	2		
281.5			4	1
283.7		2		
284.9		1	2	
287.7		1	2	1
289.0	3	3	1	1
290.5		1	2	1
291.6		2		3
293.5	1	2	2	3
294.3		3	1	
295.8	1		2	
298.2	2	6	3	8
298.8		3		
300.3	2	9	3	9
301.1	5	1		
303.7	1	4	1	
305.2				2
307.1			1	1
308.3			1	
309.5				1
311.1		2		
312.3	4	2	4	
313.5			6	5
314.8	1			
316.9				1

DECEMBER 2015	Coniferous forests		Deciduous forests	
OTUs (bp)	Invaded (n = 15)	Uninvaded (n = 16)	Invaded (n = 17)	Uninvaded (n = 17)
319.0			2	1
328.1		1	1	
331.1	3	1	2	
332.3	1	2		1
337.6			3	4
341.1	1	1	1	
345.2				1
347.2			1	4
355.2		1		
360.5	3	1		
361.5	1			
368.8		1		
417.9		2		
419.2				1
424.7	3		3	
433.1	1			
435.2	1			
444.0	1	2		1
445.8				1
459.4			4	
TOTAL	90	81	99	91

Table S8

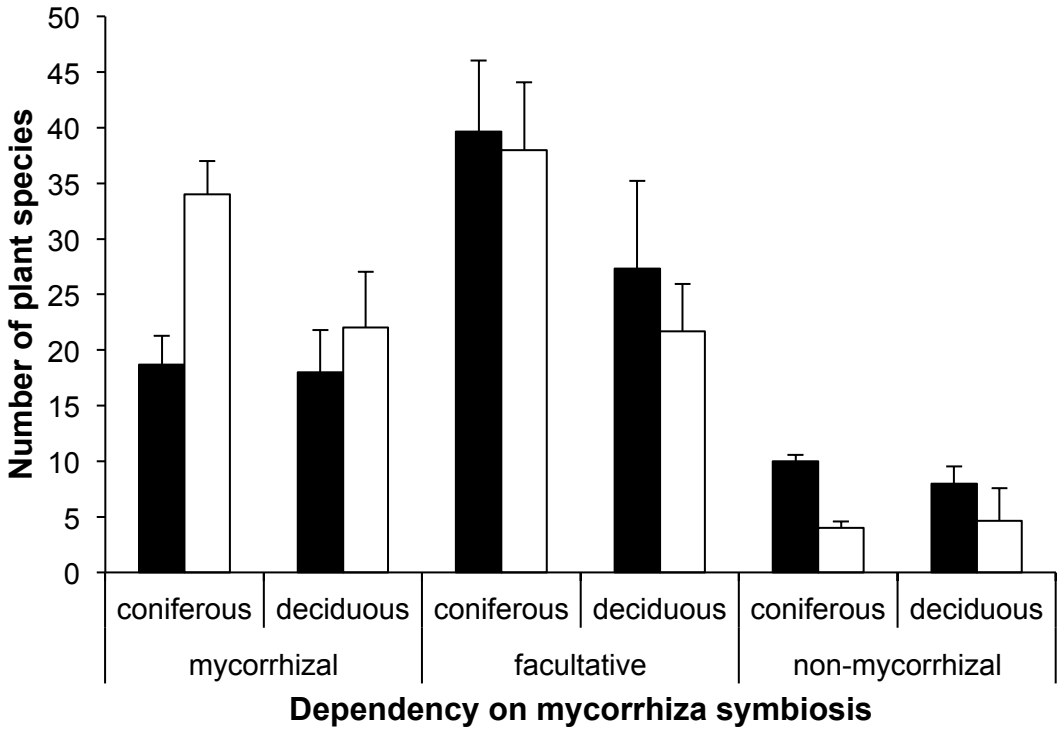
Summary of multiple regression analyses showing the effects of the density and height of *Impatiens glandulifera* on soil fungal diversity (number of operational taxonomic units) assessed at plot level in invaded areas (n = 6, with 6 plots per areas) in both coniferous and deciduous forest stands near Brislach (Basel-Landschaft), Switzerland. Cover and biomass of *I. glandulifera* were excluded from the models because of intercorrelations

	June 2015	July 2015	September 2015
Height of *I. glandulifera*	$F_{1,26} = 0.23$, $P = 0.637$	$F_{1,27} = 3.33$, $P = 0.079$	$F_{1,24} = 1.35$, $P = 0.257$
Density of *I. glandulifera*	$F_{1,26} = 0.53$, $P = 0.474$	$F_{1,27} = 8.34$, $\mathbf{P = 0.008}$	$F_{1,24} = 0.28$, $P = 0.602$

Significant P values ($P < 0.05$) are printed in bold

Fig. S1

Number of plant species subdivided according to their dependency on mycorrhiza symbiosis, and weighted by their number of occurrences in plots in areas invaded by *Impatiens glandulifera* (black bars) and in uninvaded areas (open bars) in coniferous and deciduous forest stands. Data based on Harley & Harley (1987) and Wang & Qiu (2006). Means ± SE are shown (n = 3 areas per forest type)



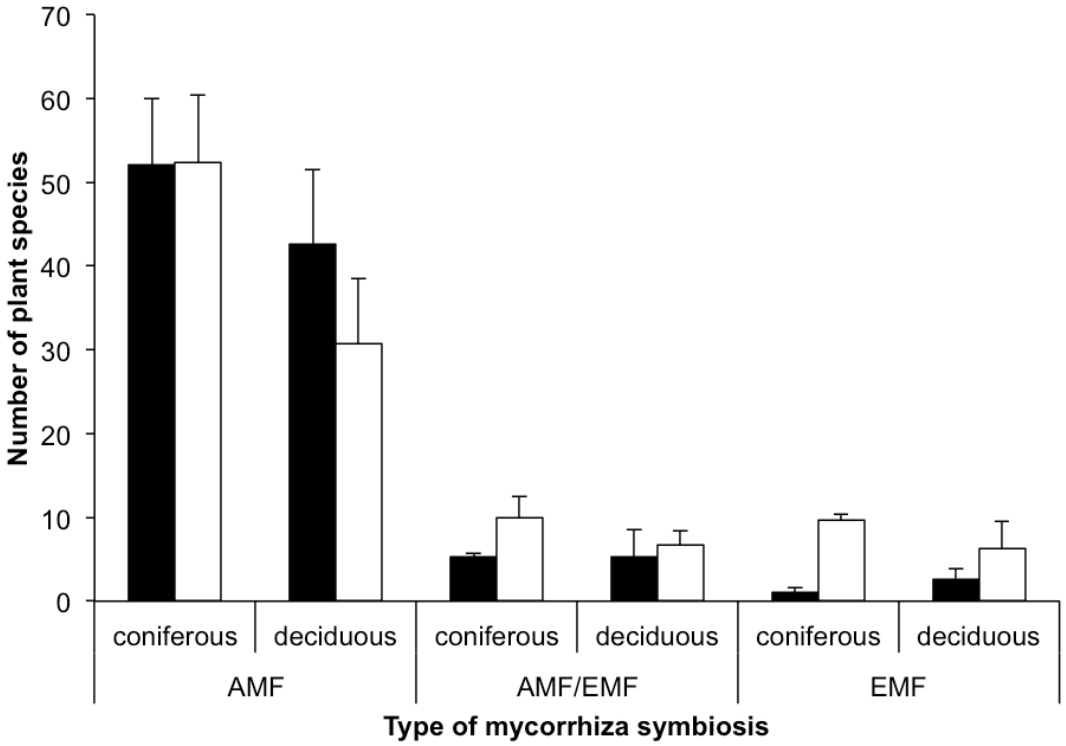
References:

Harley, J.L., Harley, E.L., 1987. A check-list of mycorrhiza in the British flora. *New Phytologist*. 105, 1–102.

Wang, B., Qiu, Y.L., 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*. 16, 299–363.

Fig. S2

Number of plant species subdivided according to the type of mycorrhiza symbiosis, and weighted by their number of occurrences in plots in areas invaded by *Impatiens glandulifera* (black bars) and in uninvaded areas (open bars) in coniferous and deciduous forest stands (AMF = arbuscular mycorrhiza; EMF = ectomycorrhiza). Data based on Harley & Harley (1987) and Wang & Qiu (2006). Means ± SE are shown (n = 3 areas per forest type)



References:

Harley, J.L., Harley, E.L., 1987. A check-list of mycorrhiza in the British flora. *New Phytologist*. 105, 1–102.

Wang, B., Qiu, Y.L., 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*. 16, 299–363.

Chapter III

The annual invasive plant *Impatiens glandulifera* reduces hyphal biomass of soil fungi in deciduous forests

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The annual invasive plant *Impatiens glandulifera* reduces hyphal biomass of soil fungi in deciduous forests

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ABSTRACT

Soil fungi play a crucial role in ecosystem functioning and there is increasing evidence that exotic plants invading forests can affect soil fungal communities. We examined potential effects of the invasive plant *Impatiens glandulifera* on hyphal biomass of ectomycorrhizal fungi, their genetic diversity and the diversity of other soil fungi in deciduous forests in Switzerland. We compared invaded patches with patches where *I. glandulifera* had been removed, by establishing pairs of 3-m long transect lines at the edge of seven areas of either type. Along the transects we assessed the length of ectomycorrhizal fungal hyphae using the 'ingrowth mesh bag method', and used terminal restriction fragment length polymorphism (T-RFLP) analysis to examine fungal genetic diversity. The invasive plant reduced fungal hyphal biomass by 30–80%: the reduction was largest in the centre of the patch. *I. glandulifera* did not alter fungal richness, but affected the composition of fungal communities. This is probably the result of a decrease of mycorrhizal fungi, coupled with an increase of saprotrophic fungi. Our findings demonstrate the adverse impacts of an annual invasive plant species on both fungal hyphal biomass and the composition of soil fungal communities. This may negatively affect forest nutrient and carbon cycling, soil stability and the functionality of the fungal community, with major consequences for forest ecosystem functioning.

1. Introduction

Soil fungi fulfil a variety of ecosystem functions (Christensen, 1989; Dighton, 2016). Considering different fungal functional types, saprotrophic fungi play a key role in decomposition and nutrient mineralization; pathogenic fungi regulate plant primary production and animal populations, and mycorrhizal fungi develop intimate and mutualistic partnerships with the roots of the majority of plant species and in this way play a crucial role in determining the diversity of plant communities and their succession dynamics (Allen, 1991; Smith and Read, 2008). In terms of biomass, mycorrhizal fungi also represent the main part of soil fungi (Nehls, 2008). Arbuscular mycorrhiza (AM) and ectomycorrhiza (EM) constitute the most abundant types of mycorrhizal symbioses. Both increase soil nutrient and water uptake of host trees, strengthen pathogen and drought resistance and thus increase the stability of forest ecosystems (Smith and Read, 2008; Courty et al., 2010). EM fungi are also essential contributors to nutrient and carbon cycling (Smith and Read, 2008). More than 50% of the annual primary production can be allocated to EM fungi (Simard et al., 2003), and biomass production and turnover of EM fungal mycelia represent a large influx to the soil carbon cycle.

Mycorrhizal fungi can occur in the soil in the form of extramatrical mycelia, which are part of the so called 'mycorrhizal network', defined as fungal hyphae that connect the roots of at least two plants (Newman, 1988; van der Heijden and Horton, 2009; Gorzelak et al., 2015). Mycorrhizal networks may cover areas ranging from a few square metres up to several hundred square metres and are able to redistribute limited resources (e.g. water, carbon, nitrogen, phosphorus) among individuals of different plant species (Smith and Read, 2008; Barto et al., 2012; Simard et al., 2012; Horton, 2015). Furthermore, mycorrhizal networks have an essential function in the regeneration of disturbed forests (Onguene and Kuyper, 2002; Booth and Hoeksema, 2010). For these reasons, the importance of extramatrical mycelia and mycorrhizal networks to plant and fungal ecology, as well as their role for ecosystem functioning, is receiving increased attention (Bever et al., 2010; Barto et al., 2012; Burke, 2012; Simard et al., 2012).

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1754-5048/

Abbreviations

AM	arbuscular mycorrhiza
EM	ectomycorrhiza
LME	linear mixed-effect model
OTU	operational taxonomic unit
T-RFLP	terminal restriction fragment length polymorphism

Extramatrical mycelia can be influenced by abiotic factors including soil temperature (Hawkes et al., 2008) and nitrogen deposition, which have negative effects on their production (Nilsson and Wallander, 2003; de Witte et al., 2017). Furthermore, biotic factors such as plant species richness and composition (Zak et al., 2003; Johnson et al., 2004) or non-native invasive species may negatively influence the formation of extramatrical mycelia (Cantor et al., 2011). These effects may be even more pronounced in the future due to the on-going climate warming, which promotes the establishment of non-native invasive species originating from regions with warmer climate (Hellmann et al., 2008). However, knowledge of the potential impact of invasive plants on extramatrical mycelia and the spatial scale in which invasive plants potentially disturb them is scarce (for an exception see Wolfe et al., 2008). Along 30 cm-long transect lines across the edges of *Alliaria petiolata* patches, Wolfe et al. (2008) found that the invasive plant inhibits EM fungal root colonization also outside the patches, probably as a result of the volatility of allelochemicals released.

The aim of our study was to investigate the spatial extent in which an invasive plant affects the biomass of mycorrhizal fungi and their diversity in forests. We focused on *Impatiens glandulifera* Royle (Himalayan balsam), a herbaceous annual plant, which is native in the western Himalaya and was introduced as a garden ornamental plant to Europe and North America in the middle of the 19th century (Beerling and Perrins, 1993). *I. glandulifera* became naturalized and invasive in riparian and disturbed habitats (Hejda and Pyšek, 2006). In the last decades, *I. glandulifera* has increasingly invaded deciduous and coniferous forests, owing to natural and man-related disturbances (Nobis, 2008; Wagner et al., 2017). The species has been classified as an invasive alien species of European Union concern by the European commission in 2017 (European Union, 2017). *I. glandulifera* can cause slight changes in plant species richness and shifts in plant species composition in riparian habitats (Hejda and Pyšek, 2006; Diekmann et al., 2016), besides promoting soil erosion along riparian zones (Greenwood and Kuhn, 2014; Greenwood et al., 2018). According to Rusterholz et al. (2017), *I. glandulifera* negatively affects both the aboveground vegetation and the soil seed bank in forests with a delay of about 15 years after the invasion. *I. glandulifera* is known to affect various aspects of underground ecosystems: the invasive plant is able to alter physical and chemical soil characteristics in forests (Ruckli et al., 2013, 2014a; Rusterholz et al., 2014), the composition of soil invertebrate communities (Tanner et al., 2013; Rusterholz et al., 2014), and the activity of soil bacteria (Gaggini et al., 2018). Furthermore, *I. glandulifera* alters the soil fungal community in forests (Gaggini et al., 2018), and negatively affects both AM and EM symbioses of tree saplings, resulting in a higher sapling mortality and in a reduced forest regeneration (Tanner and Gange, 2013; Ruckli et al., 2014a, 2016; Pattison et al., 2016). *I. glandulifera* forms symbioses with AM fungi with a colonization of 10–90% (Štajerová et al., 2009; Tanner et al., 2014; Majewska et al., 2015; Gucwa-Przepióra et al., 2016). *I. glandulifera* seems to have stronger effects on EM than on AM fungi (Gaggini et al., 2018). Ruckli et al. (2014b) identified the allelopathic compound 2-methoxy-1,4-naphthoquinone in roots and leaves of *I. glandulifera*, which is released into the soil and has strong inhibitory effects on the growth of mycorrhizal fungi and the germination of several native herbs. This indicates that naphthoquinone release may contribute to the invasion success of *I. glandulifera* and thus supports the "novel weapons hypothesis" (Callaway and Ridenour, 2004).

In an earlier study we showed that the invasion of *I. glandulifera* causes an increase in soil fungal richness and an alteration of the fungal community composition in forests (Gaggini et al., 2018), but the spatial scale of these effects has not been assessed so far. Therefore, to assess the spatial scale at which *I. glandulifera* influences the biomass of EM hyphae and the diversity of EM fungi in deciduous forests, we used the ingrowth mesh bag method (Wallander et al., 2001, 2013; Bakker et al., 2015). This is a standard method to estimate the biomass and growth of extramatrical mycelium of EM fungi in the field. We predict that the inhibitory effects of *I. glandulifera* on EM fungi extend outside of *I. glandulifera* patches.

In particular, we hypothesize that: (1) both the biomass, expressed as hyphal length, and richness of EM fungal hyphae are low in the centre of *I. glandulifera* patches and increase from the edge outside of patches, and (2) the composition of the soil fungal community changes from the centre of *I. glandulifera* patches to the outside of the patches.

2. Material and methods

2.1. Study sites and design of the field experiment

The field experiment was conducted in two study sites situated in a mixed-deciduous forest 15 km south of Basel, Northwestern Switzerland (47°26′ N, 7°33′ E). This region has a mean annual temperature of 9.4 °C and a mean annual precipitation of 947 mm (MeteoSwiss, 2016). The forest was affected by the windstorm Lothar in 1999. *I. glandulifera* started to invade several sites shortly after the storm in spring 2000. The two study sites, invaded by *I. glandulifera,* were located within a distance of 1.4 km, at elevations of 415–425 m a.s.l. The two study sites shared similar inclination, exposition, forest age and forest management, but differed slightly in tree density and composition (see Table S1 for site characteristics).

Within each study site, we selected four investigation areas measuring 10 m × 5 m with a dense and homogeneous cover of *I. glandulifera* in spring 2016. The four areas were situated 10–20 m apart from each other. In each investigation area, we installed a 3 m-long transect line perpendicular to its border. Half of the transect line was inside the invaded area, the other half outside (hereafter referred to as 'invaded transect'; Fig. 1A). At a distance of 4–5 m from the invaded transect, we installed a second transect line, analogous to the invaded transect, but placed in a 3 m-wide strip where all *I. glandulifera* plants including their roots were removed by hand (hereafter 'removed'; Fig. 1A). To prevent the recolonization of the invasive plant in the strips with removed *I. glandulifera*, we regularly removed any emerging seedlings of *I. glandulifera* at intervals of 3 weeks over the entire study period. This approach ensured that the soil conditions in strips where *I. glandulifera* has been removed are suitable for this plant species. On both transect lines 15 points were set in the range of 150 cm in the investigation area (−150 cm) to 150 cm outside the investigation area (−150, −125, −100, −80, −60, −40, −20, 0, +20, +40, +60, +80, +100, +125, +150 cm; Fig. 1B). Thus, the study set-up consisted of 16 transect lines (8 pairs of an invaded line and a line with removed *I. glandulifera*, equally distributed in both study sites) each with 15 sampling points. During the

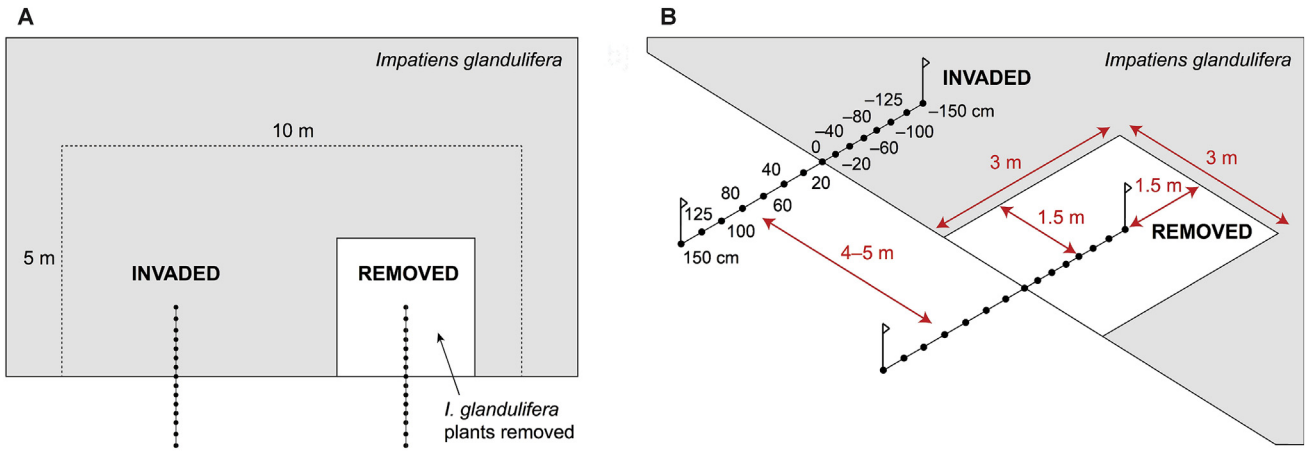

Fig. 1. Overview of an investigation area measuring 10 m × 5 m (**A**) and layout of an invaded transect line and a transect line placed in a strip where all *I. glandulifera* plants were removed (**B**).

investigation period, one pair of transect lines was destroyed by wild boar. Therefore, we excluded this pair of transect lines in the data analyses (resulting in a total of 14 transect lines and 210 sampling points). In September 2016, the density and height (cm) of *I. glandulifera* were assessed in three plots of 40 cm × 40 cm evenly distributed along the invaded transect lines.

2.2. Ingrowth mesh bag method

We used the ingrowth mesh bag method of Wallander et al. (2001) to estimate the biomass of ectomycorrhizal mycelia. According to Wallander et al. (2001) and Bakker et al. (2015), all fungal hyphae colonizing the mesh bags are considered to originate from EM fungi. Ingrowth mesh bags (9.5 cm × 4 cm x 2 cm; 50 μm mesh size) were made of nylon mesh (manufactured by Klein & Wieler oHG, Königswinter, Germany) by melting the edges together with an impulse sealer (model 205 H, Dry & Safe GmbH, Oensingen, Switzerland) and were filled with 80 g of acid-washed quartz sand (0.6–1.3 mm; Carl Roth GmbH + Co. KG, Karlsruhe, Germany). Ingrowth mesh bags had a contact area of approximately 90 cm^2 to the soil, while the mesh size of 50 μm prevented any ingrowth of plant roots. To assess the spatial impact of *I. glandulifera* on the EM hyphal biomass, the mesh bags were buried at the interface between the humus layer and mineral soil at a depth of 5–10 cm at each point of the transect lines. The mesh bags were exposed during the growing season of *I. glandulifera* from 17 May to 25 October 2016. During sample retrieval in the field, five mesh bags (2.4%) got damaged and were therefore excluded from further analyses. After sample retrieval, mesh bags were kept at 4 °C until processed.

Sample processing followed the procedure of Wallander et al. (2004) and Bakker et al. (2015) with slight modifications: each bag was opened and the sand was visually inspected for hyphal colonization in a transparent dish using a stereomicroscope. Then, the sand was placed into two lab bottles, together with 80 ml deionized water and shaken by hand for 30 s. The water was transferred with a Pipetman stepwise onto a filter paper (Whatman no. 541, diam. 90 mm) placed into a Petri dish with holes to allow the water draining from the filter. Further 40 ml deionized water were added to the sand, and the sample was processed as described above. The wet filter paper with the hyphae was placed on a regular grid (4 mm × 4 mm cells) to measure hyphal length using a stereomicroscope. Hyphal length was determined using the 'gridline intersect method' of Tennant (1975). Then, the hyphae (hereafter referred to as 'hyphal samples') were freeze-dried in Eppendorf tubes (48 h, −45 °C, VirTis BenchTop K, SP Industries Inc.) and weighed. Since the hyphal biomass in the majority of single samples was too low for genetic analyses (see below), samples at the same distance were pooled across transects in each study site, resulting in a total of 60 samples (2 invaded transect lines and 2 transect lines with removed *I. glandulifera*, each with 15 samples).

2.3. Soil sampling schedule and soil characteristics

We collected two soil samples at each point of all transect lines using a soil corer (depth 5 cm, diameter 5.05 cm, volume 100 cm^3) at the end of October 2016. The two soil samples were pooled for each point of the transect lines yielding to a total of 210 samples (15 points x 14 transect lines [invaded and removed]). Soil samples were transported on ice to the laboratory, where they were sieved (mesh size 2 mm) and a subsample was stored at −80 °C for genetic analyses (T-RFLP, see below). The remaining part of the soil samples was dried for 48 h at 60 °C. Soil moisture (%) was determined using the fresh weight to dry weight ratio. Soil pH was assessed in distilled water (1:2.5 soil:water; Allen, 1989). Total soil organic matter content (%) was determined as loss-on-ignition of oven-dried soil at 750 °C for 16 h (Allen, 1989). We did not determine total soil organic nitrogen content and total phosphorus content, because these soil characteristics were highly correlated with total soil organic matter content in a previous study conducted in the same forest (Gaggini et al., 2018).

To record soil temperature along the transect lines, Thermo Buttons (Plug and Track Proges Plus, Willems, France) were placed at a depth of 5 cm in the soil layer at each point of an invaded transect line and a transect line with removed *I. glandulifera* in one area per study site. Soil temperature was recorded at intervals of 1 h during the incubation period of the ingrowth mesh bags between 17 May and 25 October 2016. For data analyses, mean temperature averaged over the entire incubation period was used.

2.4. Fungal community profiles (T-RFLP)

T-RFLP method (terminal restriction fragment length polymorphism; Thies, 2007) was used to assess fungal community profiles, both for mycorrhizal hyphae extracted from the ingrowth mesh bags (hyphal samples), and for fungi in soil samples. The resulting number of operational taxonomic units (OTUs) was used as a measure of fungal richness in our analyses.

DNA of fungi in soil samples was extracted using the NucleoSpin Soil kit (Macherey-Nagel). DNA from hyphal samples was extracted using the DNeasy Plant Mini Kit (Qiagen). For both analyses, the internal transcribed spacer (ITS) region of fungal DNA was amplified using the primer pair ITS1-F/ITS4 (Gardes and Bruns, 1993). The procedure for PCR amplification and restriction digestion (TaqI) followed Gaggini et al. (2018). After amplification, samples were prepared as GeneScan samples (1.5 μL DNA sample, 1.5 μL GeneScan 500 LIZ size standard, 17 μL Hi-Di Formamide) and T-RFLP profiles were produced by sequencing the digests by Macrogen Inc. (Amsterdam, The Netherlands). T-RFLP analyses were successful for all 60 hyphal samples, whereas T-RFLP analyses failed in 20 (9.5%) out of 210 soil samples.

The size and the relative abundance of terminal restriction fragments were quantified using Peak Scanner software (version 1.0, Applied Biosystems, Inc.). Fragments with a size ranging from 50 to 500 bp were considered in the analyses. To avoid possible background noise, only fragments with a signal above 1% of the sum of all peak areas were included in the analyses (Li et al., 2007; Yang et al., 2016). Fragments that differed in size by 1.0 bp or less between profiles were considered as the same (Smith et al., 2005; Barto et al., 2011).

2.5. Statistical analyses

Statistical analyses were performed in R (R Foundation for Statistical Computing 2014, version 3.1.2). For all analyses, the 15 points along the transects were grouped in four distance classes (class 1: 150–80 cm in patch; class 2: 60–0 cm in patch; class 3: 20–60 cm distant to patch; class 4: 80–150 cm distant to patch). Linear mixed-effect models (LME) were used to analyse the effects of the presence of *I. glandulifera* and the distance from the patch on soil properties (moisture, total soil organic matter, pH). To avoid pseudoreplication, distance class was nested in treatment (invaded/removed), treatment was nested in transect pair, transect pair was nested in area. Treatment and distance class were included as fixed factors.

Similar LME models with nested design (see above) were applied to assess the effects of the presence of *I. glandulifera*, distance from the patch and soil characteristics on hyphal length and fungal richness (number of OTUs), both in soil samples and in hyphal samples. Soil moisture and soil pH were included as cofactors (soil organic matter content was excluded from all models because of intercorrelations; Spearman rank correlation, soil moisture: $r_s = 0.56$, $n = 210$, $P < 0.001$; soil pH: $r_s = -0.68$, $n = 210$, $P < 0.001$). Hyphal weight was highly intercorrelated with hyphal length (Spearman rank correlation: $r_s = 0.82$, $n = 188$, $P < 0.001$) and therefore was not considered in the analyses. Mesh bags that did not contain any hyphal material (8% of the samples) were excluded from all analyses. If not excluded, the analyses revealed very similar results. All models were stepwise reduced as recommended by Crawley (2007).

Multiple regression analyses were used to examine the influence of the density and height of *I. glandulifera* on hyphal length and fungal richness in soil and hyphal samples in the inner part of invaded transect lines.

Permutational multivariate analyses of variance (PERMANOVA) were used to test whether the presence of *I. glandulifera* and the distance from the patch affect fungal OTUs composition (a measure for the functionality of the fungal community), both in soil and hyphal samples (Anderson, 2005). Soil moisture and soil pH were included as cofactors. All PERMANOVA tests were based on 999 permutations of the untransformed raw data, using the *adonis* function in the *vegan* R-package. Data with presence/absence of OTUs were used in the analyses, since peak areas resulting from T-RFLP analyses are considered as an inappropriate measure for abundance due to its low reproducibility (Dickie and FitzJohn, 2007; Barto et al., 2011).

3. Results

3.1. Soil characteristics

LME analyses revealed that soil parameters did not differ between treatment or distance class along the transect, but soil moisture and total soil organic matter showed a significant interaction between treatment and distance class (Table 1; Fig. S1). Mean soil temperature was 0.5 °C lower in the inner part of invaded transects than in transects with removed *I. glandulifera* (Fig. S1).

3.2. Hyphal length along the transects

Hyphae were present in 188 mesh bags (92%). Overall mean hyphal length was 53.1 cm per bag (min. 0.3 cm, max. 369 cm), mean hyphal weight was 0.99 mg (min. <0.01 mg, max. 8.6 mg). Hyphal length inside the patch of *I. glandulifera* was reduced by 30–80% compared to transects in which the invasive species had been removed (Fig. 2A), and by 40–90% compared to the outer part of the transects (Fig. 2A). The reduction of hyphal length was largest in the centre of the patch of *I. glandulifera,* and increased linearly along the invaded transect (Fig. 2A). In contrast, there was no significant difference in hyphal length along the transects with removed *I. glandulifera* (Fig. 2A). Results of LME analyses showed that hyphal length differed between distance classes along the transect lines (Table 2), and that it decreased with increasing soil moisture (Table 2; Spearman rank correlation: $r_s = -0.32$, $n = 188$, $P < 0.001$). Multiple regression analysis showed that the density of *I. glandulifera* along the transects had a marginally significant effect on hyphal length (Table S2).

3.3. Fungal genetic diversity along the transects (T-RFLP)

T-RFLP analyses revealed a total of 183 operational taxonomic units (OTUs) in the soil samples, and 75 OTUs in the hyphal samples. Sixty-seven (89%) of the OTUs occurring in the hyphal samples also occurred in the soil samples. Therefore, we can assume that at

Table 1
Summary of linear mixed-effect model (LME) analyses showing the effects of treatment (presence of *I. glandulifera*) and distance from the patch on soil properties assessed along the transect lines.

	Soil moisture	Total soil organic matter[a]	Soil pH[b]
Treatment (invaded/removed)	$F_{1,6} = 3.57$, $P = 0.108$	$F_{1,6} = 4.51$, $P = 0.078$	$F_{1,6} = 2.12$, $P = 0.195$
Distance class	$F_{3,36} = 0.89$, $P = 0.453$	$F_{3,36} = 0.22$, $P = 0.885$	$F_{3,36} = 1.53$, $P = 0.224$
Treatment x distance class	$F_{3,36} = 5.44$, $\mathbf{P = 0.003}$	$F_{3,36} = 4.04$, $\mathbf{P = 0.014}$	$F_{3,36} = 0.40$, $P = 0.753$

Significant P values ($P < 0.05$) are printed in bold.
[a] $1/x^2$-transformed.
[b] log(x)-transformed.

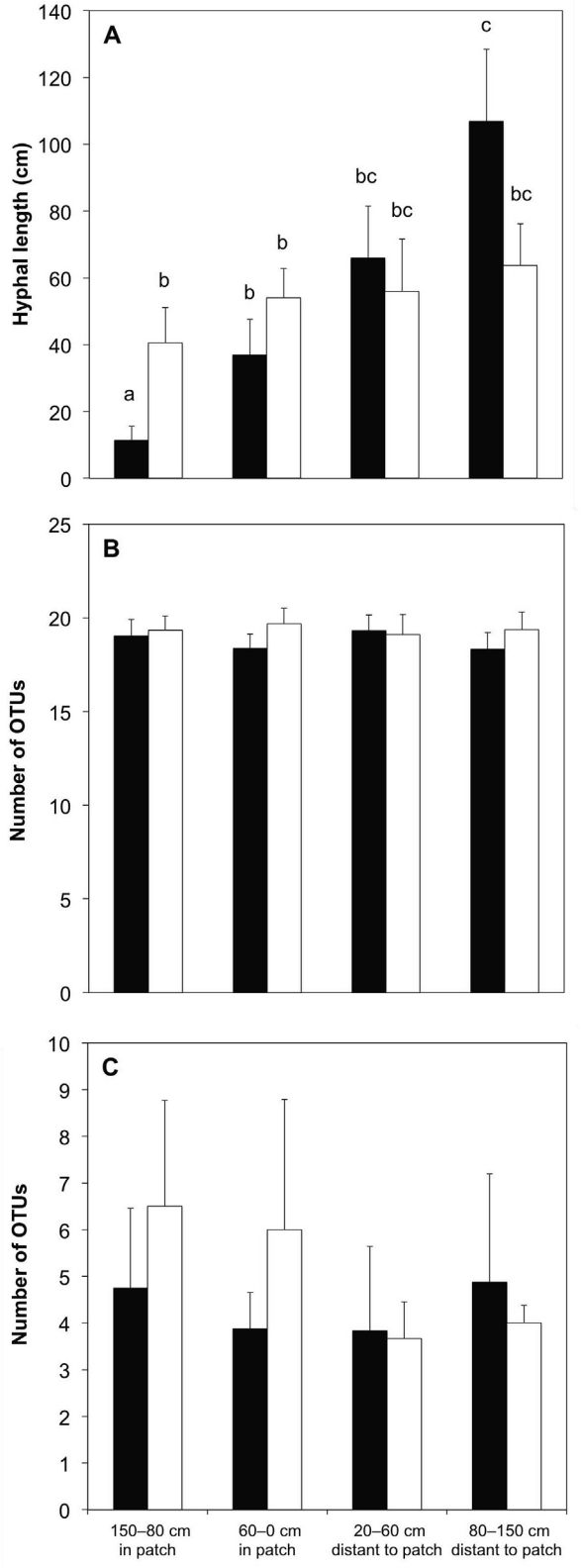

Fig. 2. (**A**) Hyphal length in ingrowth mesh bags. Number of operational taxonomic units (OTUs) as a measure of fungal diversity in (**B**) soil samples and in (**C**) hyphal samples in the four distance classes along transects invaded by *I. glandulifera* (black) and transects with removed *I. glandulifera* (white). Different letters indicate significant differences according to Wilcoxon tests, $P < 0.05$. Mean values ± SE (A,B: $n = 28$, except for class '20–60 cm distant to patch' with $n = 21$; C: $n = 8$, $n = 6$).

Table 2
Summary of linear mixed-effect model (LME) analyses showing the effects of the presence of *I. glandulifera*, distance from the patch and soil characteristics on hyphal length.

	Hyphal length[a]
Treatment (invaded/removed)	$F_{1,6} = 2.87$, $P = 0.141$
Distance class	$F_{3,36} = 6.55$, $\mathbf{P = 0.001}$
Soil moisture (%)[b]	$F_{1,130} = 5.53$, $\mathbf{P = 0.020}$
Soil pH[b]	$F_{1,130} = 1.84$, $P = 0.178$
Treatment x distance class	$F_{3,36} = 1.62$, $P = 0.202$

Significant P values ($P < 0.05$) are printed in bold.
[a] log(x+1)-transformed.
[b] sqrt(x)-transformed.

least 67 of the 183 OTUs (37%) present in the soil samples were of mycorrhizal origin. Number of OTUs in the hyphal samples was not correlated with hyphal length (Spearman rank correlation: $r_s = -0.05$, $n = 60$, $P = 0.677$).

The results of LME analyses showed that fungal richness (number of OTUs) did not differ between invaded transect lines and transects in which *I. glandulifera* had been removed, both in soil samples (Table 3; Fig. 2B) and hyphal samples (Table 3; Fig. 2C). However, the fungal richness in soil samples increased with increasing soil pH (Table 3; Spearman rank correlation: $r_s = 0.55$, $n = 190$, $P < 0.001$).

Multiple regression analysis showed that the height of *I. glandulifera* plants along the transect lines had a significant effect on fungal richness in soil samples (Table S2), whereas there was no significant influence on fungal richness in hyphal samples.

PERMANOVA analyses showed that the composition of the fungal community differed between invaded transects and transects with removed *I. glandulifera*, both in soil and hyphal samples (Table 4). Composition of the fungal community in soil samples was further influenced by soil moisture and soil pH (Table 4), whereas in hyphal samples the community composition differed between distance classes along the transects and showed a marginally significant interaction between treatment and distance class (Table 4).

4. Discussion

The present study showed that biomass of EM fungal hyphae was strongly reduced in patches invaded by *I. glandulifera* compared to patches where the invasive plant had been removed. The largest reduction in hyphal biomass was recorded in the centre of the invaded patches, and hyphal biomass increased linearly along the transect line from the centre to the outer part of the patch. Soil fungal richness in both soil samples and hyphal samples did not differ between invaded transect lines and transect lines where the invasive plant was removed, as well as between the inner and the outer part of the patch. Nevertheless, we found differences in the fungal community composition between invaded transects and transects with removed *I. glandulifera*, which can be seen as changes in the functionality of the fungal community.

4.1. Hyphal length

The reduction in hyphal length recorded in *I. glandulifera* patches is in line with the results of several studies that showed a decrease in AM and EM hyphal length in patches invaded by *Centaurea maculosa* (Mummey and Rillig, 2006) and *A. petiolata* (Cantor et al., 2011; Koch et al., 2011). The magnitude of the reduction in hyphal length (30–70%) by the annual *I. glandulifera* recorded in our study is similar or even higher to that reported in

Table 3
Summary of linear mixed-effect model (LME) analyses showing the effects of the presence of *I. glandulifera*, distance from the patch and soil characteristics on OTU's richness in soil samples and hyphal samples.

	OTUs' richness in soil samples	OTUs' richness in hyphal samples[a]
Treatment (invaded/removed)	$F_{1,6} = 3.46$, $P = 0.112$	$F_{1,1} = 1.35$, $P = 0.452$
Distance class	$F_{3,36} = 0.28$, $P = 0.837$	$F_{3,6} = 0.62$, $P = 0.626$
Soil moisture (%)[a]	–	$F_{1,42} = 1.15$, $P = 0.290$
Soil pH[b]	$F_{1,133} = 9.01$, $\mathbf{P = 0.003}$	$F_{1,42} = 1.42$, $P = 0.240$
Treatment x distance class	$F_{3,36} = 0.26$, $P = 0.853$	$F_{3,6} = 0.47$, $P = 0.715$

Significant P values ($P < 0.05$) are printed in bold.
[a] log(x)-transformed.
[b] sqrt(x)-transformed.

Table 4
Summary of PERMANOVA analyses testing the effects of the presence of *I. glandulifera*, distance from the patch and soil characteristics on fungal composition (presence/absence of OTUs) in soil and hyphal samples.

	OTUs' composition in soil samples	OTUs' composition in hyphal samples
Treatment (invaded/removed)	$F_{1,180} = 3.07$, $\mathbf{P = 0.001}$	$F_{1,50} = 2.50$, $\mathbf{P = 0.004}$
Distance class	$F_{3,180} = 1.07$, $P = 0.205$	$F_{3,50} = 2.24$, $\mathbf{P = 0.002}$
Soil moisture (%)	$F_{1,180} = 11.7$, $\mathbf{P = 0.001}$	$F_{1,50} = 4.55$, $P = 0.110$
Soil pH	$F_{1,180} = 35.8$, $\mathbf{P = 0.001}$	$F_{1,50} = 5.01$, $P = 0.515$
Treatment x distance class	$F_{3,180} = 1.17$, $P = 0.115$	$F_{3,50} = 1.40$, $P = 0.068$

Significant P values ($P < 0.05$) are printed in bold.

other studies investigating the effects of biennial (37%; Cantor et al., 2011) and perennial invasive species (24%; Mummey and Rillig, 2006). To our knowledge, no prior study has investigated the effects of annual invasive plants on hyphal biomass in the field so far. However, our result parallels those of Ruckli et al. (2014a, 2016), who found a lower AM and EM colonization rate (a proxy for hyphal biomass) on sapling roots of *Acer pseudoplatanus* and *Fagus sylvatica* planted in *I. glandulifera* patches compared to uninvaded patches.

We compared fungal biomass in invaded transects with that in transect lines in which *I. glandulifera* had been removed. Our approach ensures that the soil conditions are suitable for the establishment of the invasive plant. This may not necessarily be true for not invaded areas.

In our study, hyphal length increased linearly from the centre of the *I. glandulifera* patch towards the outer, uninvaded part (Fig. 2A), indicating that the effects of the invasive plant also extend outside the patches. A similar pattern was found by Wolfe et al. (2008) for the invasive *A. petiolata*, affecting EM fungal biomass also outside of invaded patches. In our study, we estimate that the adverse effects of *I. glandulifera* extend to a distance of 20–60 cm from the patch's edge, but this estimation is not very precise because of the linear increase of hyphal length along invaded transects (Fig. 2A). The observed pattern could also be the result of a density-dependent influence of *I. glandulifera* along the transect line. This explanation is supported by the results of the multiple regression analyses, with a weak effect of the density of *I. glandulifera* on hyphal length (Table S2). Furthermore, the *I. glandulifera* population might be older in the centre of a patch, indicating that the soil in the patch centre has been exposed to secondary compounds for a longer time. In a bioassay, Ruckli et al. (2014b) showed an inhibitory effect of the naphthoquinones released by *I. glandulifera* on the mycelium growth of three EM fungal species, as well as on the germination of native forest herbs.

The inhibitory effect of *I. glandulifera* on soil fungi 20–60 cm beyond the edges of the patch, as well as on seeds of other species (Ruckli et al., 2014b; Rusterholz et al., 2017), may facilitate the expansion of the invasive plant, because competition by other native plants is weakened. In forests, *I. glandulifera* occurs frequently in scattered patches. Compared with a single large patch, many small patches have a much larger edge along which the invasive plant can influence soil fungi and seeds. This could have pronounced effects on natural forest regeneration and, subsequently, on forest economy (Ruckli et al., 2014a, 2016).

The observed decrease of hyphal length with increasing soil moisture contradicts the findings of Bakker et al. (2015), who reported that EM hyphal production in forests was positively affected by soil moisture, but supports previous findings that the influences of soil parameters on fungal communities are an indirect effect of the *I. glandulifera* invasion (Gaggini et al., 2018). Furthermore, several studies have shown that this invasive plant can significantly change soil characteristics, for example by increasing soil moisture and soil pH in invaded areas (Ruckli et al., 2013, 2014a; Rusterholz et al., 2014).

4.2. Fungal genetic diversity (T-RFLP)

The lack of effects of *I. glandulifera* on the richness of the soil fungal community in the present study contradicts our expectations based on previous results of Gaggini et al. (2018), who found an increase in soil fungal richness in plots invaded by *I. glandulifera*. This increase was presumably a result of an increase of saprotrophic fungi, which are responsible for the fast decomposition of large amount of decaying *I. glandulifera* plants in late autumn. A possible reason for the contrasting results could be the small spatial scale (3 m-long transect lines) in the present study. When only the part of the transect lines in the *I. glandulifera* patches are considered, then multiple regression analyses showed that fungal richness increased with the height of *I. glandulifera* plants, which can be considered as a proxy for their biomass (Table S2). Plant height was largest in the centre of the patch and lowest at the patch's edge (data not shown).

The fact that the OTUs' richness in fungal hyphae was not related to hyphal length indicates that all occurring OTUs in the ingrowth mesh bags were determined in our study. The finding that fungal richness in soil samples was higher than in hyphal samples can be explained by the fact that fungal DNA extracted from soil samples represents the entire soil fungal community including saprotrophic fungi, whereas DNA extracted from hyphal samples is expected to originate only from EM fungi (Wallander et al., 2001; Bakker et al.,

2015). Therefore, we assume that in our soil samples, 37% of the OTUs were of ectomycorrhizal origin.

Differences in the fungal community composition in soil samples between invaded transect lines and transects in which *I. glandulifera* had been removed could be explained by an alteration in the proportion of saprotrophic vs. mycorrhizal fungi, which is in line with our findings in an earlier study (Gaggini et al., 2018). We hypothesize a decrease of mycorrhizal fungi, which are negatively affected by the invasive plant, coupled with an increase of saprotrophic fungi, which are responsible for the decomposition of decaying *I. glandulifera* biomass in late autumn. In hyphal samples, the alteration in the fungal community composition could be explained by the presence of more generalist mycorrhizal species surviving in the *I. glandulifera* patch, and the presence of more specialized ones in the transect lines with removed *I. glandulifera* and outside the patches. Soil fungi fulfil different functions (Christensen, 1989; Dighton, 2016), and the recorded changes in the fungal community composition in areas invaded by *I. glandulifera* may represent alterations in the functionality of the fungal community, with possible consequences for forest ecosystem functioning.

It is important to note that *I. glandulifera* is an annual species. Therefore size and spatial arrangement of its patches can change from year to year (L. Gaggini, field observation), depending also on the weather conditions of a particular year. It is possible that an area considered as 'not invaded' in the sampling year, was actually covered by *I. glandulifera* 1 y before or several years ago, and that the soil biota are still affected by the former invasion. This could explain the weak effects on fungal richness and composition found in our study. Furthermore, there could also be a delay in the effects: changes in hyphal length can be seen as the result of a current inhibition of the invasive plant (short-term effect); changes in fungal richness and composition are the results of the history of the study site (long-term effect). Changes in the proportion of saprotrophs could be the result of decomposition of *I. glandulifera* biomass from the previous winter, whereas changes in the mycorrhizal community could be the result of the invasion history of the sites in the past years. Ruckli et al. (2016) confirmed this hypothesis by showing that the diversity of EM fungi colonizing roots of *F. sylvatica* saplings declined with time after plantation in an *I. glandulifera* patch.

5. Conclusions

The invasion of the annual plant *I. glandulifera* in forests resulted in a decrease in biomass of extramatrical mycelia, which in turn may negatively affect the communication and exchange of resources between trees done by mycorrhizal networks. This would not only reduce tree fitness and ecosystem functions (van der Heijden and Horton, 2009; Simard et al., 2012), but also be problematic in light of the decreasing trends in phosphorous nutrition and growth of forest trees in Central Europe (Talkner et al., 2015; Braun et al., 2017). Moreover, the reduction in EM hyphal biomass may have consequences on the carbon cycling in forests and may even enforce the effect of climate change, because mycorrhizal fungi represent an important carbon sink and can help to offset the release of greenhouse gases to the atmosphere (Simard et al., 2003; Treseder and Holden, 2013). Fungal hyphae play an important role in the stabilization of soil by penetrating between soil particles and acting as a web to physically retain them (Tisdall, 1994). A decrease in hyphal biomass would therefore decrease soil stability in general, and increase the risk of soil erosion (Mummey and Rillig, 2006; Rillig and Mummey, 2006; Majewska et al., 2018). In fact, previous studies showed that *I. glandulifera* increases the risk of soil erosion in riparian habitats (Greenwood and Kuhn, 2014; Greenwood et al., 2018). To summarize, the impact of this annual invasive plant on forest ecosystems is not negligible. In fact, our study provides evidence that *I. glandulifera* can reduce hyphal biomass of ectomycorrhizal fungi and, even if it did not alter fungal richness, it changed the composition of the fungal community. In this way, *I. glandulifera* may compromise the stability of forest soils, alter the nutrient and carbon cycling, and affect the functionality of the fungal community. To avoid adverse effects on forest diversity and ecosystem functioning, it is highly recommended to remove the invasive plant in the early stage of its invasion.

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Supplementary data

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Supplementary material Chapter III

Table S1

Characteristics of the two 25 m x 25 m study sites invaded by *Impatiens glandulifera* located in a forest near Basel, Northwestern Switzerland

	Study site A	Study site B
Number of shrub species	5	3
Number of shrubs	23	22
Shrub height (cm, mean)	186	150
Number of tree species	7	2
Number of trees [a]	23	6
Tree diameter at breast height (cm, median)	28.0	36.6
Cover of the above-ground vegetation (%)	90	95
Cover of *Impatiens glandulifera* (%)	70	78
Cover of leaf litter (%)	7	4

[a] Study site A: 8 *Alnus glutinosa*, 6 *Fraxinus excelsior*, 4 *Fagus sylvatica*, 2 *Corylus avellana*, 1 *Acer pseudoplatanus*, 1 *Carpinus betulus*, 1 *Picea abies*
Study site B: 5 *Fagus sylvatica*, 1 *Prunus avium*

Table S2

Summary of multiple regression analyses showing the effects of the density and height of *Impatiens glandulifera* on hyphal length and fungal OTUs' richness in soil and hyphal samples inside the patches

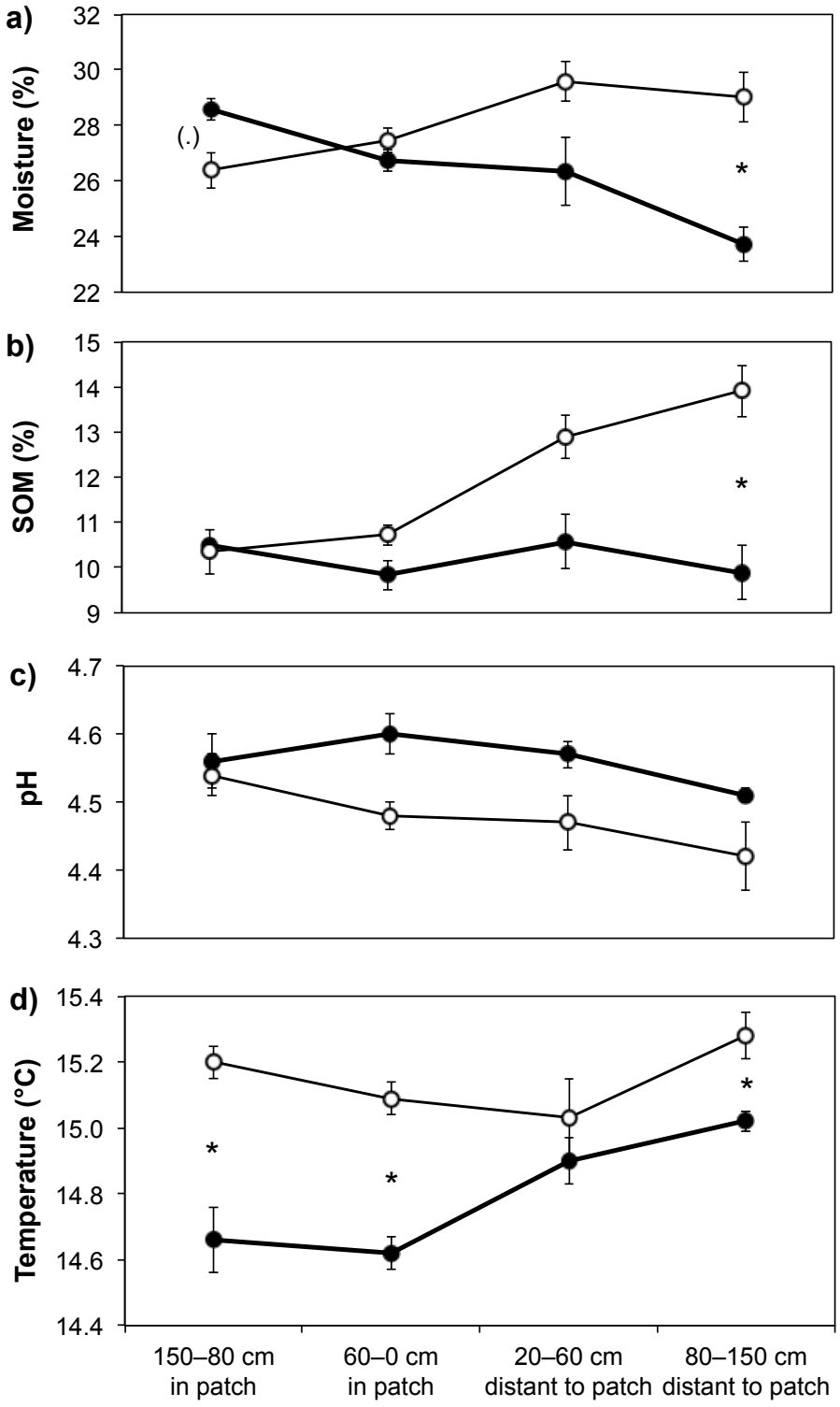
	Hyphal length [a]	OTUs' richness in soil samples	OTUs' richness in hyphal samples [a]
Density of *I. glandulifera*	$F_{1,52} = 3.16$, $P = 0.081$	$F_{1,49} = 0.01$, $P = 0.935$	$F_{1,13} = 0.04$, $P = 0.846$
Height of *I. glandulifera* (cm)	$F_{1,52} = 0.44$, $P = 0.510$	$F_{1,49} = 5.12$, $\mathbf{P = 0.028}$	$F_{1,13} = 1.04$, $P = 0.326$

Significant P values ($P < 0.05$) are printed in bold

[a] log(x+1)-transformed

Fig. S1

Soil characteristics in the four distance classes along the invaded transects (black) and control transects (white). (**a**) Soil moisture (%), (**b**) Total soil organic matter (SOM, %), (**c**) Soil pH, (**d**) Soil temperature (°C). Mean values ± SE. * $P < 0.05$, *(.)* $P < 0.10$, results from Wilcoxon tests ($n = 4$, except for class '20–60 cm distant to patch' with $n = 3$)



Chapter IV

The invasion of an annual exotic plant species affects the above- and belowground plant diversity in deciduous forests to a different extent

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The invasion of an annual exotic plant species affects the above- and belowground plant diversity in deciduous forests to a different extent

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ABSTRACT

Invasive plant species can significantly affect native biodiversity and ecosystem functioning. Even though the majority of ecosystems have more than 50% of the plant biomass belowground, most studies investigating the effects of invasive species on plant diversity focus on the aboveground vegetation. DNA-based methods allow the determination of belowground plant structures. Using these techniques, we examined potential effects of the annual invasive plant *Impatiens glandulifera* on both the above- and belowground plant species richness and composition in mixed deciduous forests in Northwestern Switzerland. We established 24 plots in three forest areas invaded by *I. glandulifera* and in three adjacent forest areas, which were not yet invaded. In each plot, we determined plant species richness and abundance in the aboveground vegetation, and collected soil samples at depths of 0–10 cm and 11–20 cm to determine belowground plant species richness. We extracted DNA from fine roots in the soil samples and applied the FAFLP technique (fluorescent amplified fragment length polymorphism) for two different regions of the chloroplast DNA (*trnL-trnF* intergenic spacer and *P6 loop*). We established a reference library for all plant species occurring in the study areas to identify the species present in mixed-root samples. Our results showed that *I. glandulifera* caused shifts in both the above- and belowground plant species composition. Plant species richness was reduced by 30% aboveground in invaded plots, but not belowground in the same plots. Many geophytes and woody species were found belowground but not aboveground in invaded plots. Root biomass was reduced by 35–55% in invaded plots, most probably due to allelopathic compounds released by the invasive plant into the soil. Our field survey shows that above- and belowground plant communities respond differently to the invasion of an annual plant species, and that the invasive species can negatively affect forest ecosystem functions, by reducing root biomass and altering plant species richness and composition.

1. Introduction

Forests cover nearly one third of the global land area, and over 80% of the world's terrestrial biodiversity can be found in forest habitats (United Nations, 2011). The diversity of the various taxonomical groups is essential for maintaining ecosystem functions in forests and delivers services, which are of immense importance to both society and the environment (Aerts and Honnay, 2011). In particular, plant diversity positively affects ecosystem functioning, including productivity and stability of forests (Vilà et al., 2007; Paquette and Messier, 2011; Chisholm et al., 2013). These relationships are based on aboveground vegetation data. However, more than 50% of plant biomass is allocated belowground in the majority of ecosystems (Jackson et al., 1997; Poorter et al., 2012; Wilson, 2014). As emphasized by Aerts and Honnay (2011), acquiring insights in the relationship between belowground biodiversity and their role for forest functioning is one of the most urgent research needs.

In the near future, it is expected that climate change, nitrogen deposition and invasive species may alter the ecosystem services provided by forests (Lindner et al., 2010; Compton et al., 2011; Vilà and Hulme, 2017). Invasive species have the potential to affect soil properties (Raizada et al., 2008), reduce the aboveground plant diversity and/or change plant species composition (Hejda et al., 2009; Vilà et al., 2011; Stoll et al., 2012), alter the composition and activity of soil microorganisms (Kourtev et al., 2002; Wolfe and Klironomos, 2005; McCary et al., 2016) and disrupt symbiotic associations between plants and soil fungi (Traveset and Richardson, 2014; Grove et al., 2017). However, the potential impact of invasive plant species on the belowground plant

Abbreviations: cpDNA, chloroplast DNA; FAFLP, fluorescent amplified fragment length polymorphism; LME, linear mixed-effect model

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species richness has not yet been assessed (for an exception see Li et al., 2018). In the past, any determination of belowground plant species richness has been hindered by difficulties of assigning roots and rhizomes to species. During the past decade, however, a number of DNA-based methods were established which allow a determination of belowground plant species richness in the field (Mommer et al., 2011; Hiiesalu et al., 2012; Pärtel et al., 2012; Rewald et al., 2012 and references therein). The majority of these methodological studies were conducted in grasslands. Forest ecosystems are still underrepresented in studies on belowground plant diversity (for exceptions see Jones et al., 2011; Randall et al., 2014; Zeng et al., 2015).

Plant invasions may differ in their impact on above- and belowground plant diversity. For example, Balogianni et al. (2014) showed a decoupling of above- and belowground responses in invaded grasslands. Furthermore, mechanisms driving plant species composition (e.g. competition) may differ in their importance for above- and belowground communities (Lamb et al., 2009; Price et al., 2012). Sampling belowground allows finding dormant plant parts or plant species that already completed their life cycle at the moment of the aboveground vegetation surveys (e.g. spring geophytes). Moreover, roots experience a longer growing season and occupy larger areas than shoots, and can persist over multiple growing seasons (Schenk and Jackson, 2002; Wilson, 2014).

To our knowledge, no study has investigated the effects of invasive plants on belowground plant diversity in deciduous forests. In this study, we focused on *Impatiens glandulifera* Royle (Himalayan balsam), an herbaceous annual plant, which is native in the western Himalaya and was introduced as garden ornamental plant to Europe and North America in the middle of the 19th century (Beerling and Perrins, 1993). *I. glandulifera* became naturalized and invasive in riparian and disturbed habitats (Hejda and Pyšek, 2006). In the last decades, *I. glandulifera* has increasingly invaded deciduous and coniferous forests, owing to natural and man-related disturbances (Nobis, 2008; Wagner et al., 2017). The species has been classified as an invasive alien species of Union concern by the European commission in 2017 (European Union, 2017). *I. glandulifera* is able to alter physical and chemical soil characteristics in forests (Ruckli et al., 2013, 2014a; Rusterholz et al., 2014; Gaggini et al., 2018) and to affect the composition of soil invertebrate communities (Tanner et al., 2013; Rusterholz et al., 2014). It can also disturb soil fungal communities and mycorrhizal symbioses (Tanner and Gange, 2013; Ruckli et al., 2014a, 2016; Pattison et al., 2016; Gaggini et al., 2018, 2019). Ruckli et al. (2014b) identified the allelopathic compound 2-methoxy-1,4-naphthoquinone in roots and leaves of *I. glandulifera*, which is released into the soil and has strong inhibitory effects on the growth of mycorrhizal fungi and the germination of several native herbs. This supports the "novel weapons hypothesis" (Callaway and Ridenour, 2004) and indicates that naphthoquinone release may contribute to the invasion success of *I. glandulifera*. Allelopathic compounds seem also to reduce plant root elongation (Schenk et al., 1999).

Studies investigating the effects of *I. glandulifera* on aboveground plant species richness and composition yielded contradictory results, both in forests and river banks. Some studies revealed a negative influence of *I. glandulifera* on native plant species richness (Maule et al., 2000; Hulme and Bremner, 2006; Diekmann et al., 2016; Rusterholz et al., 2017), while other studies showed that the invasive plant did not affect plant species richness of native communities (Hejda and Pyšek, 2006; Hejda et al., 2009; Tanner et al., 2013; Künzi et al., 2015; Čuda et al., 2017; Gaggini et al., 2018). Furthermore, some studies showed shifts in plant species composition after the invasion of *I. glandulifera* (Tanner et al., 2013; Diekmann et al., 2016; Čuda et al., 2017; Gaggini et al., 2018). These contradictory results may be the consequence of differences in habitat and soil types, and/or invasion history. In particular, Rusterholz et al. (2017) provided experimental evidence for a delayed response of about 15 years of both the aboveground vegetation and the soil seed bank to the invasion of *I. glandulifera* in forests.

The aim of our study was to assess the potential effect of *I. glandulifera* on the belowground plant diversity by comparing both above- and belowground plant species richness in invaded and uninvaded deciduous forests. We established plots in forest areas with dense stands of *I. glandulifera* and plots in adjacent forest areas that were not yet colonized by the invasive plant. We conducted vegetation surveys to record aboveground plant species richness, and collected root samples to assess belowground plant species richness. To do this, we applied the fluorescent amplified fragment length polymorphism analysis (FAFLP), according to Taggart et al. (2011) and Karst et al. (2015). In particular, we hypothesized that 1) both above- and belowground plant species richness are reduced in plots invaded by *I. glandulifera* compared to uninvaded forest plots, and 2) the reduction in plant species richness is less pronounced belowground than aboveground, because species might survive for a longer period in form of root or rhizome belowground than aboveground. Furthermore, we hypothesized that 3) root biomass is lower in invaded than in uninvaded plots.

2. Materials and methods

2.1. Study sites and design of the survey

The study was conducted in three sites in a mixed-deciduous forest 15 km south of Basel, Northwestern Switzerland (47°26′ N, 7°33′ E). This region has a mean annual temperature of 9.4 °C and an annual precipitation of 947 mm (MeteoSwiss, 2016). The forest was affected by the windstorm Lothar in 1999. *I. glandulifera* started to invade several sites shortly after the storm in spring 2000. The three study sites were located within an area of 1.4 km × 0.8 km, with a distance of 870–1500 m (mean 1100 m) between each other, at elevations of 410–425 m a.s.l. In spring 2017, we selected a 25 m × 25 m area invaded by *I. glandulifera* (hereafter referred to as 'invaded area') in each study site. An area of the same size that was not yet colonized by the alien plant (hereafter referred to as 'uninvaded area') was chosen in proximity to the corresponding invaded one (mean distance between invaded and uninvaded area 15 m, range 5–25 m). Pairs of invaded and uninvaded areas shared similar elevation, inclination, exposition, soil type, forest stand type and forest age, but slightly differed in the tree density and composition (Table S1 – *Electronic Supplementary Material*). Two study sites (No. 1 and 3) were colonized by *I. glandulifera* before 2008, the other (No. 2) in 2011. Thus, at the time of our study, the sites were invaded since 6 and > 10 years. The presence of a few *I. glandulifera* individuals at the edge of the uninvaded areas (Table S1) indicates that uninvaded areas also provide suitable habitat for the invasive plant. In each invaded area, we installed four randomly chosen 4 m × 4 m plots, which had a similar cover of *I. glandulifera* (> 50%). Similarly, four 4 m × 4 m plots were installed in the corresponding uninvaded areas. This resulted in a total of 24 plots (four plots x two invasion states [invaded and uninvaded] x three sites).

2.2. Data collection

To assess the impact of *I. glandulifera* on aboveground plant diversity, the richness and abundance of vascular plant species were assessed. Each of the 4 m × 4 m plots was subdivided in four subplots (2 m × 2 m). In each subplot, all plants in the ground vegetation (herbs and woody plants up to a height of 50 cm) were determined to the species and their cover was estimated using the Braun-Blanquet (1964) scale at the end of May 2017. The same procedure was applied for the plant species belonging to the shrub layer (woody plants, with an height between 50 cm and 5 m). The total cover of the ground vegetation was visually estimated (accuracy 5%) and the mean height (cm) of the ground vegetation, as well as of *I. glandulifera* in invaded plots, was measured. Plant surveys were repeated at the beginning of September 2017 to complete plant species lists. The recorded plants were classified according to Lauber et al. (2012). Tree canopy closure above each plot

was assessed using photographs and determined with the pixel counting function of Adobe Photoshop (version 10.0.1).

To characterize the forest community of the invaded and uninvaded areas in the three study sites, we subdivided each area into five 5 m × 25 m stripes. In each of the five stripes, all plant species belonging to the shrub layer (50 cm to 5 m height) and to the tree layer (height > 5 m) were recorded and the number of individuals for each species were counted in September 2017 (Table S1). We also visually estimated the total vegetation cover of the ground vegetation (herb layer), as well as the cover of leaf litter, bare ground and that of *I. glandulifera* (accuracy 5%) in each stripe. The stem diameter (cm) of each individual tree was measured at breast height (DBH).

To assess the local plant species pool, we recorded all plant species occurring in each of the invaded and uninvaded areas (25 m × 25 m) during the growing season, from April to October 2017, checking at monthly intervals for additional species, to ensure not to overlook ephemeral species. For each plant species we collected some leaf material from 2 to 3 individuals, which was preserved through silica gel desiccation and used for genetic analyses (see below).

Furthermore, we collected three randomly chosen soil samples using a soil corer (depth 20 cm, diameter 5.15 cm, volume 416 cm^3; separated in two layers, 0–10 cm and 11–20 cm) in each 2 m × 2 m subplot. Soil samples were transported on ice to the laboratory. One soil sample was used to assess soil characteristics (see below). The other two soil samples were mixed and used for determining the belowground plant diversity. This resulted in a total of 192 samples (24 plots x 4 subplots x 2 soil depths [0–10 cm and 11–20 cm]).

For the assessment of the belowground plant diversity, we washed the soil samples with tap water using a sieve (mesh size 1 mm) and collected the fine roots (diameter < 3 mm) present in a standardized way. Searching effort was 20 min per sample and only fine root fragments longer than 3 mm were collected. Dead roots were not considered. Root material was then put in Eppendorf tubes. To assess the weight of fine roots, samples were freeze-dried using a laboratory freeze-drier (48 h, −45 °C, VirTis BenchTop K, SP Industries Inc.) and then weighed. Freeze-dried roots were pulverized using a ball mill (Retsch type MM 200; Retsch GmbH, Haan, Germany) for 10 min at 30.0 Hz and then used for genetic analyses (see below).

2.3. Soil characteristics

Soil samples were sieved (mesh size 2 mm) and dried for 48 h at 60 °C. Soil moisture (%) was determined using the fresh weight to dry weight ratio. Soil pH was assessed in distilled water (1:2.5 soil:water; Allen, 1989). Total soil organic matter content (%) was determined as loss-on-ignition of oven-dried soil at 750 °C for 16 h (Allen, 1989). We did not determine total soil organic nitrogen content and total phosphorus content, because these soil characteristics were highly correlated with total soil organic matter content in a previous study conducted in the same forest (Gaggini et al., 2018).

2.4. Genetic analyses (FAFLP)

DNA from both the leaf samples collected in each study area and root samples collected at subplot level was extracted using DNeasy Plant Mini Kit (Qiagen), following manufacturer's instructions (except for adding 4 µL Proteinase K to every sample at the beginning). For leaf and root samples, 5 mg and 10 mg, respectively were used as starting material for the DNA extraction. Foliar DNA was used to generate a FAFLP size key (library) to determine the belowground species richness of mixed root samples (see below). Following Taggart et al. (2011), three non-coding regions of chloroplast DNA (cpDNA) were independently amplified with fluorescently labelled universal primers (Taberlet et al., 1991, 2006): 1) the *trnL* intron with primers C (5' CGAAATCGGTAGACGCTACG) and D (5'GGGGATAGAGGGACTTG AAC), 2) the *trnL-trnF* intergenic spacer with primers E (5'GGTTCAAG TCCCTCTATCCC) and F (5'ATTTGAACTGGTGACACGAG), and 3) the *P6 loop* of the *trnL* intron with primers G (5'GGGCAATCCTGAGCCAA) and H (5'CCATTGAGTCTCTGCACCTATC). The frequently used primer pair AB (*trnT-trnL* intergenic spacer; Taberlet et al., 1991) was tested, the PCR conditions slightly modified, but finally excluded because of failure in the PCR amplification of several samples. This was also reported by Taggart et al. (2011), Randall et al. (2014) and Karst et al. (2015), and following their advices, the primer pair AB was replaced by the primer pair GH (Taberlet et al., 2006). One primer per each pair was labelled with a different fluorescent dye (primer C: ATTO532 as alternative to VIC; primer E: ATTO550 as alternative to NED; primer G: FAM; Microsynth AG, Balgach, Switzerland), to enable the simultaneous visualization of all three regions.

PCR reactions (25 µL) consisted of 5 µL of template DNA (5–10 ng), 12.5 µL Master mix (HotStarTaq Master Mix Kit, Qiagen), 2.5 µL of each primer (10 µM), 0.5 µL BSA (1 µg/µL) and 2 µL sterile water. PCR amplification was achieved in an Eppendorf Mastercycler Pro (Vaudaux-Eppendorf AG, Schönenbuch, Switzerland). Each region had unique thermal cycler conditions: 1) *trnL* intron (primers CD), 95 °C 15 min, 2 cycles of 94 °C 60 s, 60 °C 60 s, 72 °C 80 s, followed by 33 cycles of 94 °C 45 s, 59.6–0.4 °C/cycle 60 s, 72 °C 80 s and a final extension of 72 °C for 30 min; 2) *trnL-trnF* intergenic spacer (primers EF), 95 °C 15 min, 2 cycles of 94 °C 60 s, 60 °C 60 s, 72 °C 80 s, followed by 33 cycles of 94 °C 45 s, 63–0.4 °C/cycle 60 s, 72 °C 80 s and a final extension of 72 °C for 30 min; 3) *P6 loop* (primers GH), 95 °C 15 min, 35 cycles of 95 °C 30 s, 55 °C 30 s, 72 °C 120 s, and a final extension of 72 °C for 30 min. Products were visualized by gel electrophoresis (1.5% agarose gel) to check for successful amplification. For each primer pair, the included negative controls revealed no contamination throughout all PCR reactions.

Prior to fragment analysis, the amplified products from the three loci were diluted (1:100), mixed together, and then prepared as GeneScan samples (1 µL mixed DNA sample, 1 µL GeneScan 1200 LIZ size standard, 18 µL Hi-Di Formamide). Fragment analysis was conducted by Macrogen Inc. (Amsterdam, The Netherlands), by resolving the pooled PCR products on a capillary sequencer (ABI 3730 XL DNA analyser). The size of the fluorescent amplified fragments was quantified using Peak Scanner software (version 1.0, Applied Biosystems, Inc.). According to Taggart et al. (2011), fragment sizes obtained by the capillary sequencer were rounded to the nearest base pair and, to account for potential error in sizing by the capillary sequencer, fragment lengths with a range of the reported value ± 1 bp were used for the analyses. We excluded the *trnL* intron region from all analyses, because of frequent failures in the fragment analysis (33% of the mixed root samples). In the other two regions (*trnL-trnF* intergenic spacer and *P6 loop*), fragment analysis only failed in 4% and 25%, respectively, of the samples. Failed samples were equally distributed over treatment and soil depths.

Following the recommendation of Karst et al. (2015), we checked whether the likelihood of fragment detection was influenced by fragment size. However, we found no size-based fragment competition in our study (*trnL-trnF* intergenic spacer, 0–10 cm soil depth: $r_s = 0.02$, $n = 74$, $P = 0.857$, 11–20 cm: $r_s = 0.01$, $n = 74$, $P = 0.965$; *P6 loop*, 0–10 cm: $r_s = 0.02$, $n = 74$, $P = 0.841$, 11–20 cm: $r_s = -0.06$, $n = 74$, $P = 0.626$).

2.5. FAFLP size key

Data from leaf samples were used to generate a multilocus barcode library (FAFLP size key) of the two markers (*trnL-trnF* intergenic spacer and *P6 loop*) for all plant species found in the invaded and uninvaded study areas. In the case of leaf samples, PCR amplification and fragment analysis were replicated three times for each sample, to ensure consistency and accuracy of amplicon sizes. Leaf material from 2 to 3 individuals was included to account for within-species variation. Because of the sensitivity of the method, multiple peaks were observed from

Table 1
Reference library based on FAFLP analysis for the plant species recorded in the aboveground vegetation of our study sites, with their fragment sizes (bp) for the two investigated cpDNA regions.

Family	Species	*trnL-trnF* intergenic spacer	*P6 loop* (*trnL* intron)
Adoxaceae	*Sambucus nigra* L.	441	82–83
	Sambucus racemosa L.	450	82–83
	Viburnum opulus L.	458	71–72
Apiaceae	*Aegopodium podagraria* L.[a]	446	76–77
	Angelica sylvestris L.[a]	444	77–78
	Sanicula europaea L.	432–433	76–77
Araceae	*Arum maculatum* L.	458	56–57
Araliaceae	*Hedera helix* L.	450	78–80
Asparagaceae	*Polygonatum multiflorum* (L.) All.	432	86–87
Asteraceae	*Cirsium oleraceum* (L.) Scop.[b]	447	78–79
	Cirsium palustre (L.) Scop.[b]	447	78–79
	Solidago virgaurea L.	419	58–59
	Taraxacum officinale aggr.	403–404	82–83
	Unknown (Asteraceae 1)	479	81
Balsaminaceae	*Impatiens glandulifera* Royle	452–453	89–90
Betulaceae	*Alnus glutinosa* (L.) Gaertn.[c]	467–468	94–95
	Betula pendula Roth	477–479	94–95
	Carpinus betulus L.[c]	469–470	93–95
	Corylus avellana L.	472–473	93–95
Brassicaceae	*Cardamine amara* L.	634	81–82
	Cardamine flexuosa aggr.	692	81–82
	Cardamine pratensis aggr.	650	81–82
Campanulaceae	*Phyteuma spicatum* L.	433	80–81
Caprifoliaceae	*Lonicera xylosteum* L.	442	78–79
Caryophyllaceae	*Cerastium* sp.	458	80–81
	Moehringia trinervia (L.) Clairv.	417	65–66
	Silene dioica (L.) Clairv.	479	81–82
Cyperaceae	*Carex acutiformis* Ehrh.[d]	431–432	116–117
	Carex leporina L.	457	115–116
	Carex pallescens L.[d]	421–422	116–117
	Carex pendula Huds.[d]	425	116–117
	Carex remota L.[d]	436–437	115–116
	Carex sylvatica Huds.[d]	432	116–117
Dryopteridaceae	*Dryopteris dilatata* aggr.	344	73–74
	Dryopteris filix-mas (L.) Schott	353	73–74
Fabaceae	*Lotus pedunculatus* Cav.	251–252	89–90
	Vicia sepium cf.	438–440	59
Fagaceae	*Fagus sylvatica* L.	277	97–98
	Quercus robur L.[e]	462–463	86–87
	Quercus rubra L.[e]	462–463	87–88
Geraniaceae	*Geranium robertianum* L.	471	86–87
Hypericaceae	*Hypericum humifusum* L.	234–235	80–81
	Hypericum perforatum L.	271–272	58–59
Juncaceae	*Juncus effusus* L.	394–395	83–84
	Luzula pilosa (L.) Willd.	343	83–84
Lamiaceae	*Ajuga reptans* L.	380	74–75
	Galeopsis tetrahit L.	373–374	75
	Glechoma hederacea L.	371–372	77–78
	Lamium galeobdolon s.l.[d]	372	76
	Lamium maculatum (L.) L.	371	74–75
	Stachys sylvatica L.	376	74–75
Melanthiaceae	*Paris quadrifolia* L.	456	87–88
Oleaceae	*Fraxinus excelsior* L.	420	71–72
	Ligustrum vulgare L.	496	83–84
Onagraceae	*Circaea lutetiana* L.	460–461	84–85
	Epilobium hirsutum cf.[f]	357–358	86–87
	Epilobium tetragonum cf.[f]	355–356	86–87
Oxalidaceae	*Oxalis acetosella* L.	438–439	82–83
Pinaceae	*Abies alba* Mill.	443	80–82
	Picea abies (L.) H. Karst.	462	88–90
	Pinus sp.	463–464	79–80
Plantaginaceae	*Veronica montana* L.	426	75–76
	Veronica officinalis L.	409	75–76
Poaceae	*Agrostis gigantea* Roth	389	86–87
	Brachypodium sylvaticum (Huds.) P. Beauv.	429	86–87
	Festuca sp.	423	86–87
	Milium effusum L.	425–426	86–87
	Unknown (Poaceae 1)	422	91–92
	Unknown (Poaceae 2)	422	86–87
Polygonaceae	*Polygonum* sp.[d]	423	61–63
	Rumex obtusifolius L.	446	62–63
Primulaceae	*Lysimachia nemorum* L.	317	78–79
	Lysimachia vulgaris L.[d]	338–339	77–78

(*continued on next page*)

Table 1 (*continued*)

Family	Species	*trnL-trnF* intergenic spacer	*P6 loop* (*trnL* intron)
	Primula elatior (L.) L.	456–457	75–77
Ranunculaceae	*Anemone nemorosa* L.	502–503	83–84
	Caltha palustris L.	458	84–85
	Ranunculus ficaria L.	492	81–82
	Ranunculus repens L.	470	75–76
Rosaceae	*Crataegus laevigata* (Poir.) DC.[g]	490–491	83–85
	Filipendula ulmaria (L.) Maxim.	472	90–91
	Fragaria vesca L.	505	85–86
	Geum urbanum L.	477	85–86
	Potentilla sterilis (L.) Garcke	474	85–86
	Prunus avium L.[h]	466–467	84–85
	Prunus padus L.	434–435	84–85
	Prunus spinosa L.[h]	467–468	84–85
	Rubus sp.[g]	490	85–86
Rubiaceae	*Galium aparine* L.	428	64–65
	Galium odoratum (L.) Scop.	439–440	64–65
Sapindaceae	*Acer pseudoplatanus* L.	437–438	89–90
Urticaceae	*Urtica dioica* L.	444	66–67
Violaceae	*Viola reichenbachiana* Boreau	438	82–83
Woodsiaceae	*Athyrium filix-femina* (L.) Roth	392	73–74

[a,b,c,e,f,g,h] Grouped in the analyses to artificial species complexes, due to similar fragment sizes in both regions.
[d] Omitted from the analyses following the criteria of Hiiesalu et al. (2012).

single samples. Since our goal was to find unique identifiers for each plant species, we selected peaks that had the largest peak area and occurred in all three replicates (Table 1). The three replicates of each of the 93 plant species recorded in our study revealed identical fragment sizes, indicating a high fragment size reproducibility. Moreover, the results of a pre-test confirmed that fragment sizes did not differ between roots and leaves, by applying the same technique to roots and leaves from 8 different species. Because amplicon sizes of both cpDNA regions were similar for some congenerics and/or confamilials, these species were grouped to artificial species complexes in the analyses (n = 80 taxonomic units; see footnotes in Table 1). The same species complexes were used for the aboveground data.

We used the created FAFLP size key to identify which plant species occurred in the mixed root samples. We established presence-absence lists following Karst et al. (2015), who recommended that the FAFLP method should solely be used to demonstrate the presence of a plant species and not to quantify its abundance. According to Taggart et al. (2011), different criteria can be used to indicate the potential presence of a species: the approach can be unconstrained (using the entire species pool based on all study sites) or constrained (the species pool is limited to only those species found in the single study site). As suggested by Taggart et al. (2011), we used the constrained approach for the following reasons: 1) the unconstrained approach frequently gives rise to false positives (detection of species known to be absent; Taggart et al., 2011), and 2) the species pool for the single study sites (pairs of invaded and uninvaded areas) was based on observations made over the entire growing season and in a large area around the study plots. Furthermore, the approach can follow a conservative (all known species peaks must be detected to record species presence) or a liberal identification criterion (only one known species peak must be detected to assign species presence). In our analyses, we applied the conservative criterion. Furthermore, eight of 76 species that occurred aboveground within the plots could not be detected belowground by the FAFLP analysis (*Carex acutiformis*, *C. pallescens*, *C. pendula*, *C. remota*, *C.*

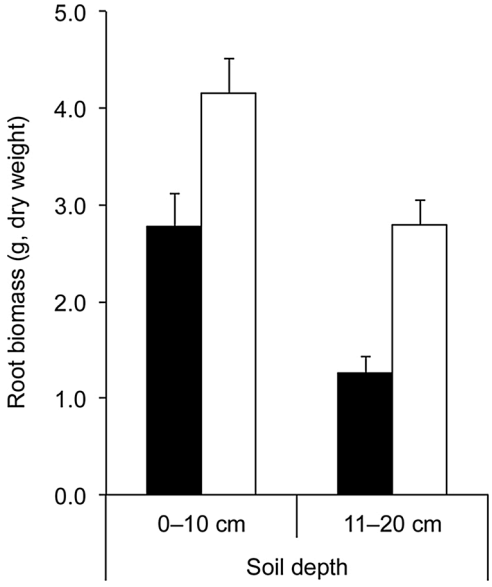

Fig. 1. Total fine root biomass (g dry weight in 3.33 dm^3 soil volume; soil depth 0–10 cm and 11–20 cm) assessed at plot level in areas invaded by *I. glandulifera* (black bars) and in uninvaded areas (white bars). Means ± SE are shown (n = 12 in each case).

Table 2
Summary of linear mixed-effect model (LME) analyses testing the effects of treatment (presence/absence of *I. glandulifera*) and soil depth on various soil properties.

	Soil moisture	Soil pH[a]	Total soil organic matter[a]
Invasion status (presence of *I. glandulifera*)	$F_{1,2}$ = 41.42, **P = 0.023**	$F_{1,2}$ = 0.13, P = 0.751	$F_{1,2}$ = 8.65, P = 0.100
Soil depth (0–10 cm / 11–20 cm)	$F_{1,22}$ = 81.72, **P < 0.001**	$F_{1,23}$ = 9.09, **P = 0.006**	$F_{1,23}$ = 91.16, **P < 0.001**
Invasion status x soil depth	$F_{1,22}$ = 4.97, **P = 0.036**	–	–

Significant P values (P < 0.05) are printed in bold.
– Excluded from the model after step-wise reduction.
[a] (1/x)-transformed.

Table 3
Summary of linear mixed-effect model (LME) analyses testing the effects of treatment (presence/absence of *I. glandulifera*), soil depth and soil characteristics on root biomass and on two measures of belowground plant species richness.

	Root biomass	Total belowground plant species richness	Additional belowground plant species richness
Invasion status (presence of *I. glandulifera*)	$F_{1,2} = 41.53$, $\boldsymbol{P = 0.023}$	$F_{1,2} = 1.20$, $P = 0.388$	$F_{1,2} = 30.50$, $\boldsymbol{P = 0.031}$
Soil depth (0–10 cm / 11–20 cm)	$F_{1,23} = 42.20$, $\boldsymbol{P < 0.001}$	$F_{1,20} = 5.51$, $\boldsymbol{P = 0.029}$	$F_{1,19} = 5.03$, $\boldsymbol{P = 0.037}$
Soil moisture (%)	–	–	$F_{1,19} = 5.27$, $\boldsymbol{P = 0.033}$
Soil pH[a]	–	$F_{1,20} = 8.36$, $\boldsymbol{P = 0.009}$	$F_{1,19} = 19.14$, $\boldsymbol{P < 0.001}$
Invasion status x soil depth	–	–	–

Significant P values ($P < 0.05$) are printed in bold.
– Excluded from the model after step-wise reduction.
[a] (1/x)-transformed.

sylvatica, *Lamium galeobdolon* s.l., *Lysimachia vulgaris* and *Polygonum* sp.). Following Hiiesalu et al. (2012), these species were omitted from all the analyses.

2.6. Data analyses

Statistical analyses were performed in R (R Foundation for Statistical Computing 2014, version 3.1.2). For all analyses, subplot data were pooled to plot level (mean soil characteristics and total species number). Linear mixed-effect models (LME) were used to analyse the effects of the presence of *I. glandulifera* and soil depth on soil properties (moisture, total soil organic matter, pH). To avoid pseudoreplication, plot was nested in treatment (invaded/control), and treatment was nested in area. Treatment and soil depth were included as fixed factors.

Following Hiiesalu et al. (2012), we enumerated three aspects of species richness: 1) aboveground plant species richness, sampled visually during the vegetation surveys; 2) total belowground plant species richness (aboveground richness of species rooted in the plot plus additional belowground richness detected by DNA analysis); 3) additional belowground species richness (species detected by DNA analysis of mixed root samples but absent in the aboveground vegetation survey). LME models with nested design (see above) were applied to assess the effects of the presence of *I. glandulifera*, soil depth (in the case of belowground richness) and soil properties on aboveground, total belowground and additional belowground plant species richness. Soil moisture and soil pH were included as cofactors (soil organic matter content was excluded from all the models because of intercorrelation with soil moisture; $r_S = 0.51$, $n = 48$, $P < 0.001$). All models were stepwise reduced as recommended by Crawley (2007).

Permutational multivariate analyses of variance (PERMANOVA) were used to test whether the presence of *I. glandulifera* affects above- and belowground plant species composition (Anderson, 2005). Soil moisture, soil pH and aboveground vegetation cover were included as cofactors. All PERMANOVA tests were based on 999 permutations of the untransformed raw data, using the *adonis* function in the *vegan* R-package. Presence/absence data of species were used in the analyses.

Analysis of covariance (ANCOVA) was used to examine potential differences between invaded and uninvaded plots in the relationship between aboveground and total belowground species richness.

3. Results

3.1. Soil characteristics

At both soil depths, soil moisture was 50% higher in plots invaded by *I. glandulifera* than in uninvaded plots, and total soil organic matter was 20–30% higher in invaded than in uninvaded plots (Table 2; Fig. S1). In contrast, soil pH did not differ between invaded and uninvaded plots (Table 2; Fig. S1). Results of LME analyses confirmed this finding for soil moisture, but not for total soil organic matter (Table 2). LME analyses also revealed that soil parameters differed between the two soil depths (Table 2). Furthermore, soil moisture showed a significant interaction between treatment and soil depth (Table 2).

3.2. Root biomass

Root biomass declined with soil depth (Fig. 1). It was reduced by 35% in plots invaded by *I. glandulifera* compared to uninvaded plots at 0–10 cm soil depth, and by 55% at 11–20 cm depth (Table 3; Fig. 1). Root biomass was correlated with total belowground plant species richness at 0–10 cm soil depth ($r_S = 0.41$, $n = 24$, $P = 0.050$), but not at 11–20 cm soil depth ($r_S = 0.01$, $n = 24$, $P = 0.996$).

3.3. FAFLP size key

We used the PCR amplicon sizes obtained from both the *trnL-trnF* intergenic spacer and *P6 loop* regions for all 93 plant species occurring in the study sites to generate the FAFLP size key (Table 1). The *trnL-trnF* intergenic spacer was in most cases species-specific, while the *P6 loop*

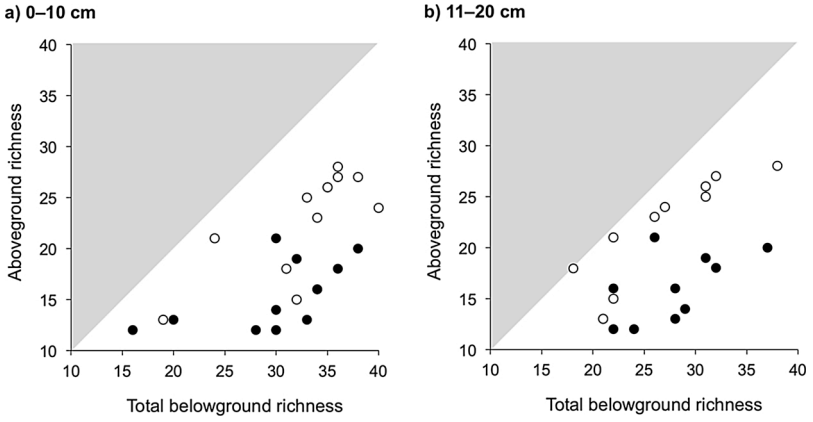

Fig. 2. Pattern of aboveground species richness (assessed in 4 m^2 plots) in relation to the calculated total belowground plant species richness (measured as the aboveground richness of species rooted in the plot plus additional belowground richness detected by DNA analysis) for the soil depth of 0–10 cm (**a**) and 11–20 cm (**b**) in the corresponding plots invaded by *I. glandulifera* (filled circles) and in uninvaded plots (open circles). The shaded area represents a region outside the operational space (aboveground richness cannot exceed total belowground richness). Please note that above- and belowground plant species richness are based on different sampling areas.

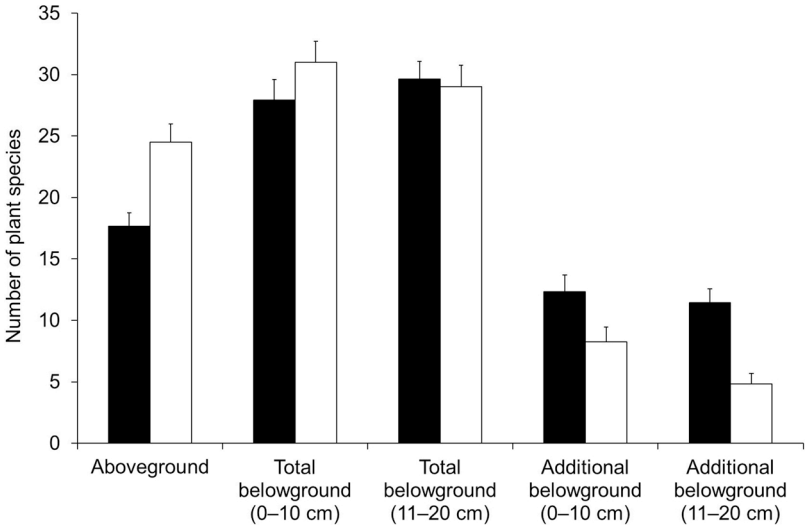

Fig. 3. Number of plant species recorded above- and belowground (soil depth 0–10 cm and 11–20 cm) at plot level in areas invaded by *I. glandulifera* (black bars) and in uninvaded areas (white bars). Additional belowground richness only includes species that were detected by DNA analysis but were absent in the aboveground vegetation survey. Means ± SE are shown (n = 12 in each case). Please note that above- and belowground plant species richness are based on different sampling areas.

Table 4
Summary of linear mixed-effect model (LME) analyses testing the effects of treatment (presence/absence of *I. glandulifera*) and soil characteristics on aboveground plant species richness.

	Aboveground plant species richness
Invasion status (presence of *I. glandulifera*)	$F_{1,2}$ = 17.95, P = 0.051
Soil moisture (%)	$F_{1,17}$ = 1.38, P = 0.256
Soil pH[a]	–

– Excluded from the model after step-wise reduction.
[a] (1/x)-transformed.

was rather discriminant at the family level. The combined use of both cpDNA regions permitted the identification of 84% of all plant species found in our study sites.

3.4. Above- and belowground plant species richness and composition

Out of a total of 87 plant species recorded in the three study sites, 49 species (56.3%) were found in the aboveground vegetation in invaded plots and 53 (60.9%) in uninvaded plots (Table S2). Belowground, at a soil depth of 0–10 cm, 69 species (79.3%) occurred both in invaded and uninvaded plots. The corresponding values for the soil depth of 11–20 cm were 66 (75.9%) and 62 (71.3%) species. The relative frequency of plant species in mixed root samples was positively correlated with their relative frequency of aboveground species (r_S = 0.57, n = 80, P < 0.001).

Species-accumulation curves showed that invaded plots harboured more plant species (10–20 species) belowground than aboveground, independently on the number of plots considered, whereas this difference was less pronounced in uninvaded plots (8–15 species; Fig. S2). Total belowground plant species richness increased with increasing aboveground species richness (Fig. 2). ANCOVA analyses with combined data for the two soil depths showed a significant relationship between above- and total belowground richness (t = 6.52, $d.f.$ = 41, P < 0.001). This relationship differed between invaded and uninvaded areas (t = –4.72, $d.f.$ = 41, P < 0.001), but not between the two soil depths (t = 1.88, $d.f.$ = 41, P = 0.068). There was no significant interaction between treatment (presence/absence of *I. glandulifera*) and soil depth.

Aboveground plant species richness was lower in invaded plots than in uninvaded plots (Fig. 3; Table 4), whereas total belowground plant species richness was not affected by the presence of the invasive plant (Fig. 3; Table 3). In contrast, additional belowground plant species richness was higher in invaded plots than in uninvaded plots at both soil depths (Fig. 3; Table 3). Both total and additional belowground species richness were higher at a soil depth of 0–10 cm than at 11–20 cm (Table 3), and decreased with increasing soil pH (total belowground species richness: r_S = –0.44, n = 46, P = 0.002; additional belowground species richness: r_S = –0.31, n = 46, P = 0.036). Additional belowground species richness was further influenced by soil moisture (Table 3).

Both above- and belowground plant species composition differed between invaded and uninvaded plots and also among study sites (Table 5). Species compositions were also influenced by soil pH and cover of the aboveground vegetation (Table 5).

4. Discussion

The present study showed that *I. glandulifera* significantly reduced

Table 5
Summary of PERMANOVA testing the effects of treatment (presence/absence of *I. glandulifera*), and soil and plot characteristics on above- and belowground plant species composition (at different soil depths). Cover data of *I. glandulifera* were not considered in the analyses.

	Aboveground plant species richness	Total belowground plant species richness (soil depth 0–10 cm)	Total belowground plant species richness (soil depth 11–20 cm)
Invasion status (presence of *I. glandulifera*)	$F_{1,23}$ = 9.44, **P = 0.001**	$F_{1,23}$ = 3.94, **P = 0.001**	$F_{1,21}$ = 4.31, **P = 0.001**
Study site	$F_{4,23}$ = 4.36, **P = 0.002**	$F_{4,23}$ = 7.26, **P = 0.001**	$F_{4,21}$ = 6.30, **P = 0.003**
Soil moisture (%)	$F_{1,23}$ = 1.67, P = 0.120	$F_{1,23}$ = 1.80, P = 0.114	–
Soil pH[a]	$F_{1,23}$ = 3.16, **P = 0.009**	$F_{1,23}$ = 2.65, **P = 0.005**	$F_{1,21}$ = 1.98, **P = 0.011**
Aboveground vegetation cover (%)	$F_{1,23}$ = 2.61, **P = 0.009**	$F_{1,23}$ = 2.42, **P = 0.050**	$F_{1,21}$ = 2.45, **P = 0.004**

Significant P values (P < 0.05) are printed in bold.
– Excluded from the model after step-wise reduction.
[a] (1/x)-transformed.

the root biomass of native plants in invaded plots. Aboveground plant species richness was lower in invaded than uninvaded plots, but in contrast to our expectations, total belowground plant species richness did not differ between invaded and uninvaded plots. *I. glandulifera* caused significant shifts in plant species composition, both above- and belowground. However, we found an asymmetry in the responses of the above- and belowground plant community to *I. glandulifera* invasion, with effects being more pronounced above- than belowground.

4.1. Root biomass

Plant root biomass decreased with soil depth, as found by Frank et al. (2010) and Kesanakurti et al. (2011). The presence of *I. glandulifera* reduced total root biomass, probably as a result of the allelopathic compounds released by the invasive plant (Schenk et al., 1999). The reduction was higher at a soil depth of 11–20 cm than at 0–10 cm. This could be caused by an infiltration and accumulation of the water-soluble naphthoquinones, which negatively affect seedling germination and root elongation (Terzi, 2008; Ruckli et al., 2014b). A further explanation for the higher reduction in root biomass at the soil depth of 11–20 cm than at 0–10 cm is that roots of *I. glandulifera* are very short and mostly accumulated in the upper soil layer (Clements et al., 2008). Thus, *I. glandulifera* roots contribute to the root biomass at 0–10 cm depth, but to a lesser extent at 11–20 cm depth, while the inhibitory effects of the naphthoquinones play a role in both soil layers. By reducing root biomass, *I. glandulifera* also reduces soil stability, an important ecosystem function (Vannoppen et al., 2015). This effect is further enforced by the lower hyphal biomass of soil fungi in the presence of the invasive plant (Gaggini et al., 2019). In fact, previous studies showed that *I. glandulifera* increases the risk of soil erosion in riparian habitats (Greenwood and Kuhn, 2014; Greenwood et al., 2018).

4.2. FAFLP size key

The two cpDNA regions *trnL-trnF* intergenic spacer (primers EF) and *P6 loop* of the *trnL* intron (primer GH) were effective at characterizing the taxonomic diversity of roots in plant communities in mixed-deciduous forests in our study in Northwestern Switzerland. The combined use of the two cpDNA regions permitted to distinguish 84% of all plant species found in our study sites, and this percentage is comparable with the results of Kress et al. (2009) and Frank et al. (2010). In our study, the likelihood of fragment detection was not influenced by fragment size. The fact that the amplification of the cpDNA regions *trnT-trnL* intergenic spacer (primers AB) and *trnL* intron (primers CD) was not successful in the majority of our samples parallels the results of several studies applying the same FAFLP method (Taggart et al., 2011; Randall et al., 2014; Karst et al., 2015).

4.3. Above- and belowground plant species richness and composition

Aboveground plant species richness was reduced in plots invaded by *I. glandulifera*. Considering our three study sites separately (*Fig. S3*), the reduction in plant species richness found in the study site No. 2 (20%) was less pronounced than in the two other sites (30–40%). This could be caused by differences in their invasion history. Indeed, site No. 2 has been invaded only 6 years before our study, whereas the other two sites were invaded more than 10 years before our study. This interpretation is supported by the findings of Rusterholz et al. (2017), who reported a time effect in the response of aboveground vegetation to the invasion of *I. glandulifera*, with a delay of about 15 years. Thus, the invasion age appears to be a key element to understand the effects of this invasive plant.

A reduced aboveground plant species richness following the invasion of *I. glandulifera* was also found by Maule et al. (2000), Hulme and Bremner (2006), Diekmann et al. (2016) and Rusterholz et al. (2017). Other studies, however, showed contradictory results (Hejda and Pyšek, 2006; Hejda et al., 2009; Tanner et al., 2013; Künzi et al., 2015; Čuda et al., 2017; Gaggini et al., 2018). In our study, the invasion of *I. glandulifera* shifted the plant species composition. Similar patterns were reported by Tanner et al. (2013), Rusterholz et al. (2017), Čuda et al. (2017) and Gaggini et al. (2018). The discrepancy between the outcomes of the various studies could be explained by differences in the invasion history and in soil and habitat characteristics. Unfortunately, most studies investigating the impact of *I. glandulifera* on plant diversity had no information on invasion age of their study areas. In general, the effects of *I. glandulifera* on plant diversity seem to be more pronounced in forests than in riparian habitats. The habitat-specific response to the invasive plant could be due to site-related differences in propagule pressure and frequency of disturbances.

Total belowground plant species richness decreased with soil depth. A similar pattern was observed by Kesanakurti et al. (2011) and Li et al. (2018). Contradicting our hypothesis, total belowground richness did not differ between invaded and uninvaded plots. It is interesting to note that there was an asymmetry between above- and belowground plant communities in their response to *I. glandulifera* invasion. Our study was the first that investigated potential effects of plant invasions on belowground plant species richness in temperate forests. In this respect, any comparisons with other studies are not possible. However, Hiiesalu et al. (2012) provided evidence that above- and belowground plant richness of grasslands responded differently to variable soil fertility. Even if aboveground plant richness declines relatively rapidly with increasing soil fertility, belowground richness might remain high for a longer period (Hiiesalu et al., 2012). Our results support the expectation of less pronounced impacts belowground than aboveground. Interspecific competition could have stronger negative effects on the aboveground vegetation, while species might survive for a longer period in a plot in form of root or rhizome belowground. Moreover, the survival rate of roots could be higher than that of shoots.

In the present study, the reduced aboveground species richness following the invasion, and the fact that total belowground species richness did not differ between invaded and uninvaded plots, resulted in a higher additional belowground richness in plots invaded by *I. glandulifera*. In particular, geophytes and phanerophytes (woody species) occurred more frequently belowground than aboveground in invaded areas (Fig. S4). In spring, the cover of *I. glandulifera* seedlings is extremely dense and geophytes suffer from a high competition for light, which negatively affects their growth. With time, it is possible that this competition is fateful for geophytes, leading to their local extinction both above- and belowground. With regard to phanerophytes, it is important to note that their growth is inhibited by the presence of *I. glandulifera*. As shown by Ruckli et al. (2014a,2016), the invasive plant has strong negative effects on mycorrhizal colonization and on the survival of tree saplings, with critical consequences on forest regeneration. In fact, in our study 7.2 ± 0.5 phanerophyte species (mean ± SE) occurred aboveground in form of seedlings or saplings in uninvaded plots, compared to 3.3 ± 0.5 species in plots invaded by *I. glandulifera*. Therefore, the detection of phanerophyte species in belowground root samples of invaded plots is mainly a result of root fragments of shrubs and trees present in the surroundings of the sampling plots.

Our study design does not allow any direct comparisons of plant diversity between above- and belowground vegetation, because aboveground plant diversity was sampled in plots of 4 m x 4 m, whereas belowground diversity was assessed in eight soil cores with 5 cm diameter and 20 cm depth. In contrast, Hiiesalu et al. (2012) explored

grassland plant richness in scales of 1000 cm^3 (10 × 10 × 10 cm) both above- and belowground. In forests this is not possible, because plant species are often clonal and patchily distributed.

5. Conclusions

This is to our knowledge the first study investigating the effects of an annual invasive plant on belowground plant species richness in forests. There is a need for more research in this topic. Our study revealed an asymmetry between the above- and belowground plant diversity in response to the invasion of *I. glandulifera*. Aboveground plant species richness was reduced by 30% in invaded plots compared to uninvaded ones, whereas total belowground species richness did not differ between invaded and uninvaded plots. Many geophytes and woody species were found below- but no longer aboveground in plots invaded by *I. glandulifera,* probably as a consequence of light competition and due to allelopathy. In the case of woody species, the low number of seedlings and saplings found aboveground in invaded plots can be problematic in future with respect to rejuvenation of invaded forests. Rusterholz et al. (2017) reported a delay of about 15 years after *I. glandulifera* invasion until the aboveground plant species richness decreased. The delay in the belowground plant species richness might be even more pronounced than in the aboveground vegetation, because roots and rhizomes can persist belowground over multiple growing seasons. In case of extirpation of the invasive annual plant, belowground plant diversity may be an important source for the successful restoration of native forest plant communities. Importantly, productivity and soil stability of forest ecosystems can be negatively affected by the invasion of *I. glandulifera*, because the invasion drastically reduces root biomass. This effect is enforced by the hyphal biomass reduction of soil fungi in the presence of the invasive plant (Gaggini et al., 2019). In general, to avoid adverse effects on forest diversity and forest ecosystem functioning, it is highly recommended to remove the invasive plant in the early stage of the invasion. Furthermore, our results demonstrate a successful application of the FAFLP technique for the assessment of belowground plant diversity in deciduous forests.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ppees.2019.04.004.

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Supplementary material Chapter IV

Table S1

Characteristics of the study areas invaded by *Impatiens glandulifera* and of uninvaded areas located in a forest near Basel, Northwestern Switzerland, assessed in September 2017

	Study site 1		Study site 2		Study site 3	
	Invaded area	Uninvaded area	Invaded area	Uninvaded area	Invaded area	Uninvaded area
Number of tree species	7	8	2	6	4	10
Number of tree stems	23	52	6	32	23	52
Tree diameter, DBH (cm, median)	28.0	14.6	36.6	23.9	37.6	8.1
Number of shrub species	5	3	3	5	3	6
Number of shrubs	23	51	22	13	42	27
Shrub height (cm, mean)	186	156	150	128	200	222
Height of ground vegetation (cm) [a]	35	36	35	28	28	32
Height of *I. glandulifera* plants (cm) [a]	152	–	127	–	138	–
Canopy closure (%) [a]	79	90	57	90	86	89
Aboveground vegetation cover (%)	90	66	95	33	99	94
Cover of *Impatiens glandulifera* (%)	70	0	78	1	75	10
Cover of leaf litter (%)	7	29	4	62	1	6
Bare ground cover (%)	3	5	1	5	0	0

[a] Assessed at plot level, mean

Table S2

List of plant species recorded in the aboveground vegetation in the study areas invaded by *Impatiens glandulifera* and in uninvaded areas located in a forest near Basel, Northwestern Switzerland; *x* = species recorded in the study plots; *(x)* = species occurring in the area, but not in the study plots

Species	Study site 1		Study site 2		Study site 3	
	Invaded area	Uninvaded area	Invaded area	Uninvaded area	Invaded area	Uninvaded area
Abies alba Mill.		x	x			x
Acer pseudoplatanus L.	x	x	x	x	x	x
Aegopodium podagraria L. [a]	(x)					
Agrostis gigantea Roth	x		x	x	x	x
Ajuga reptans L.	(x)	x		x		x
Alnus glutinosa (L.) Gaertn. [b]	(x)	(x)			(x)	(x)
Anemone nemorosa L.	x	x	x	x	x	x
Angelica sylvestris L. [a]		x		x		x
Arum maculatum L.					(x)	x
Athyrium filix-femina (L.) Roth	x	x	x	x	x	x
Betula pendula Roth			x			
Brachypodium sylvaticum (Huds.) P. Beauv.						x
Caltha palustris L.	x				x	(x)
Cardamine amara L.					(x)	
Cardamine flexuosa aggr.					x	
Cardamine pratensis aggr.						x
Carex acutiformis Ehrh. [c]	x		x		(x)	
Carex leporina L.					(x)	
Carex pallescens L. [c]			x		x	
Carex pendula Huds. [c]			x			x
Carex remota L. [c]	x		x		x	x
Carex sylvatica Huds. [c]	x	x	x	x	x	x
Carpinus betulus L. [b]	x	x	x	x	(x)	x
Cerastium sp.				x		
Circaea lutetiana L.	x	x	x	x	x	x
Cirsium oleraceum (L.) Scop. [d]					(x)	
Cirsium palustre (L.) Scop. [d]			(x)			
Corylus avellana L.	(x)	x		x	(x)	x
Crataegus laevigata (Poir.) DC. [e]					(x)	(x)
Dryopteris dilatata aggr.	x	x	x	x	x	x
Dryopteris filix-mas (L.) Schott	(x)	x	(x)	x		x
Epilobium hirsutum cf. [f]			x			
Epilobium tetragonum cf. [f]			x			
Fagus sylvatica L.	(x)	x	(x)	x	(x)	x
Festuca sp.				x		
Filipendula ulmaria (L.) Maxim.	x	x			(x)	
Fragaria vesca L.			x			
Fraxinus excelsior L.	(x)	x		x	x	x
Galeopsis tetrahit L.	(x)		x	x	(x)	
Galium aparine L.	x	x	x	x		x
Galium odoratum (L.) Scop.	(x)	x	(x)	x		x
Geranium robertianum L.	(x)	x		x		
Geum urbanum L.	x	x	x	x	x	x
Glechoma hederacea L.	x	x			x	x
Hedera helix L.	x	x	x	x	x	x
Hypericum humifusum L.			x	x		
Hypericum perforatum L.			x			
Impatiens glandulifera Royle	x	(x)	x	x	x	x

Species	Study site 1		Study site 2		Study site 3	
	Invaded area	Uninvaded area	Invaded area	Uninvaded area	Invaded area	Uninvaded area
Juncus effusus L.			x	x	x	x
Lamium galeobdolon s.l. [c]	x		x	x	x	x
Lamium maculatum (L.) L.	(x)					
Ligustrum vulgare L.		x			(x)	x
Lonicera xylosteum L.		(x)		(x)		
Lotus pedunculatus Cav.			(x)			
Luzula pilosa (L.) Willd.			x			
Lysimachia nemorum L.	(x)	(x)	(x)		x	
Lysimachia vulgaris L. [c]						x
Milium effusum L.					x	
Moehringia trinervia (L.) Clairv.	x	x	x	x	x	x
Oxalis acetosella L.	x	x	x		x	x
Paris quadrifolia L.	x	x			(x)	x
Phyteuma spicatum L.		(x)				
Picea abies (L.) H. Karst.			x	x	(x)	x
Pinus sp.				(x)		
Polygonatum multiflorum (L.) All.	(x)	x		x		x
Polygonum sp. [c]				x		
Potentilla sterilis (L.) Garcke		x				x
Primula elatior (L.) L.	x	(x)			(x)	x
Prunus avium L. [g]		x	x	x	x	x
Prunus padus L.	(x)	x				
Prunus spinosa L. [g]	(x)			x	x	x
Quercus robur L. [h]		x		x		x
Quercus rubra L. [h]			x	x		
Ranunculus ficaria L.	x	x			x	x
Ranunculus repens L.	x		(x)	x	(x)	
Rubus sp. [e]	x	x	x	x	x	x
Rumex obtusifolius L.	(x)		x			
Sambucus nigra L.		x	x	x	(x)	x
Sambucus racemosa L.	(x)		x			
Sanicula europaea L.	x					
Silene dioica (L.) Clairv.						(x)
Solidago virgaurea L.				(x)		
Stachys sylvatica L.	x	x	x		x	x
Taraxacum officinale aggr.	(x)		x	(x)		
Urtica dioica L.	x	(x)	x	x		
Veronica montana L.	x	x	x	x	x	x
Veronica officinalis L.			x	x		
Viburnum opulus L.	x	x		x	(x)	x
Vicia sepium cf.	x					
Viola reichenbachiana Boreau		x		x		x
Unknown (Asteraceae 1)						x
Unknown (Poaceae 1)				x		
Unknown (Poaceae 2)			x			
Total species number						
in study plots	31	37	42	43	28	48
in study area	49	44	49	47	47	52

[a, b, d, e, f, g, h] Grouped to artificial species complexes both in above- and belowground species lists, due to similarity in fragment sizes

[c] Not found belowground, therefore omitted from both above- and belowground species lists, following the criteria of Hiiesalu et al. (2012)

Fig. S1

Soil moisture (**a**), soil pH (**b**) and soil organic matter content (**c**) at 0–10 cm and 11–20 cm soil depth, assessed at plot level in areas invaded by *Impatiens glandulifera* (black bars) and in uninvaded areas (white bars). Means ± SE are shown (n = 12)

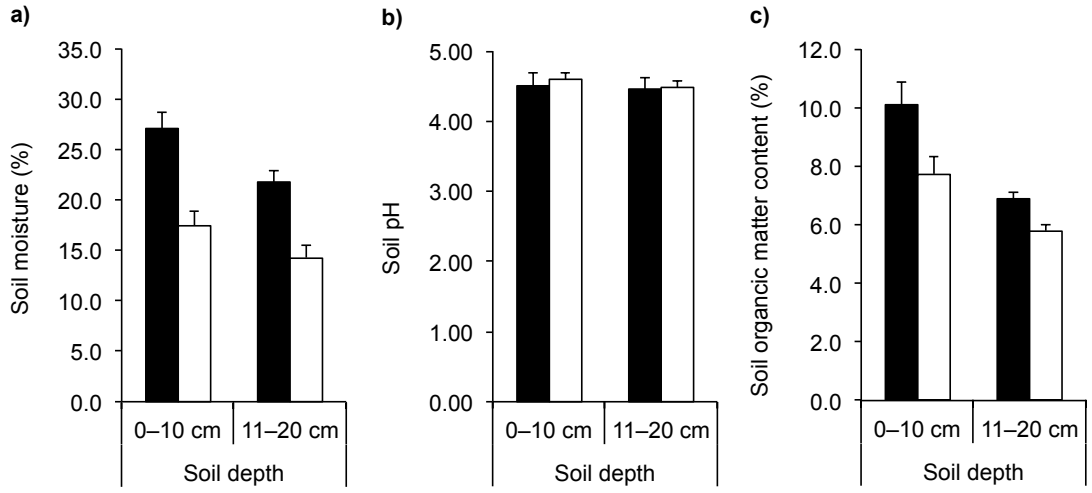


Fig. S2

Species-accumulation curves for aboveground species richness (**a**) in plots (4 m x 4 m) invaded by *Impatiens glandulifera* (filled circles) and in uninvaded plots (open circles), total belowground species richness at 0–10 cm soil depth (**b**) and total belowground species richness at 11–20 cm soil depth (**c**). The same curves are represented in a grouped way for invaded plots (**d**; bold line: above-ground species richness, thin line: total belowground species richness at 0–10 cm soil depth, dashed line: total belowground species richness at 11–20 cm soil depth) and uninvaded plots (**e**). Means ± 95% confidence intervals are shown

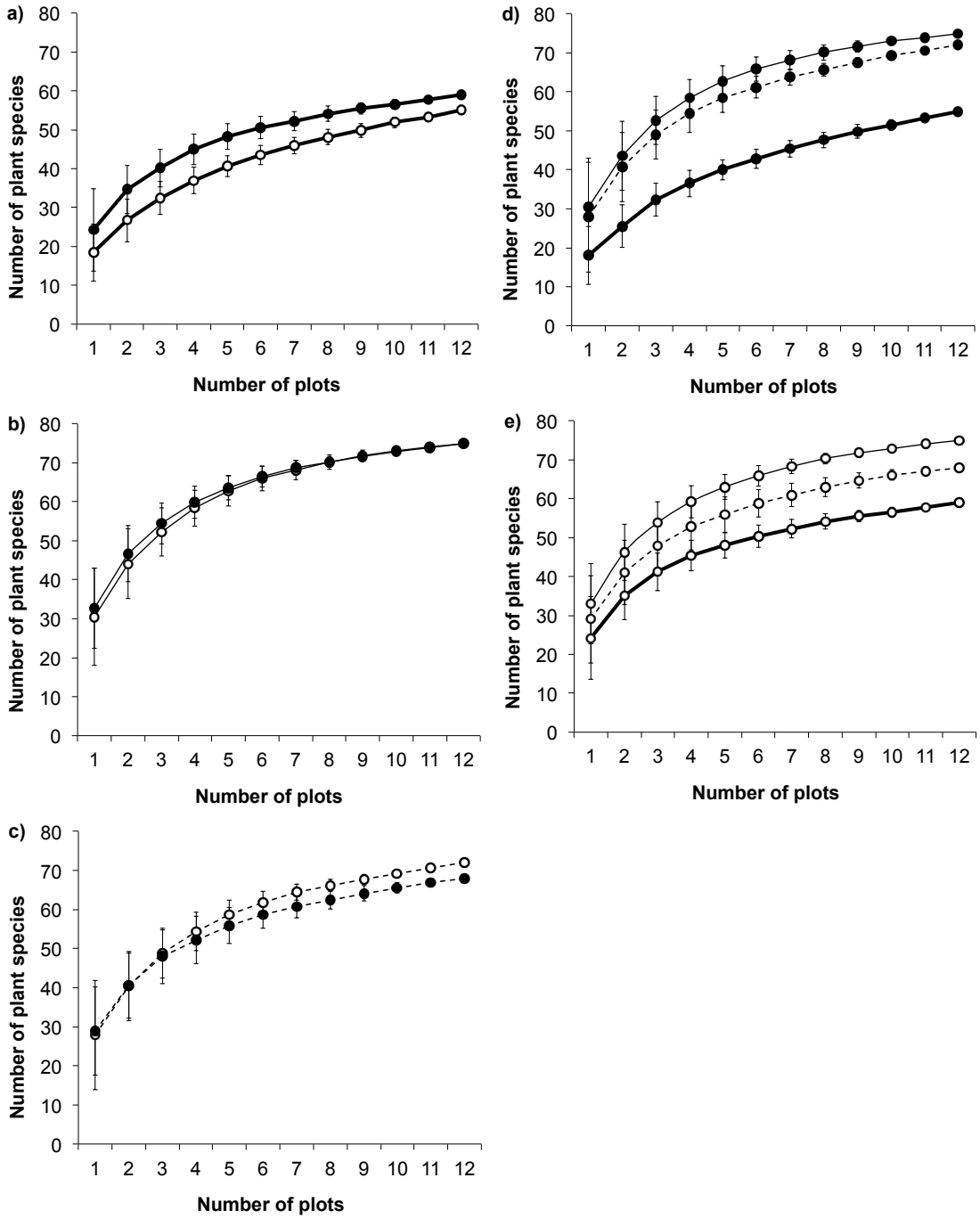


Fig. S3

Number of plant species recorded above- (**a**) and belowground (**b**: 0–10 cm and **c**: 11–20 cm soil depth) at plot level in areas invaded by *Impatiens glandulifera* (black bars) and in uninvaded areas (white bars). Additional belowground richness (**d**, **e**) only includes species that were detected by DNA analysis but were absent in the aboveground vegetation survey. Means ± SE per study site are shown (n = 4 in each case)

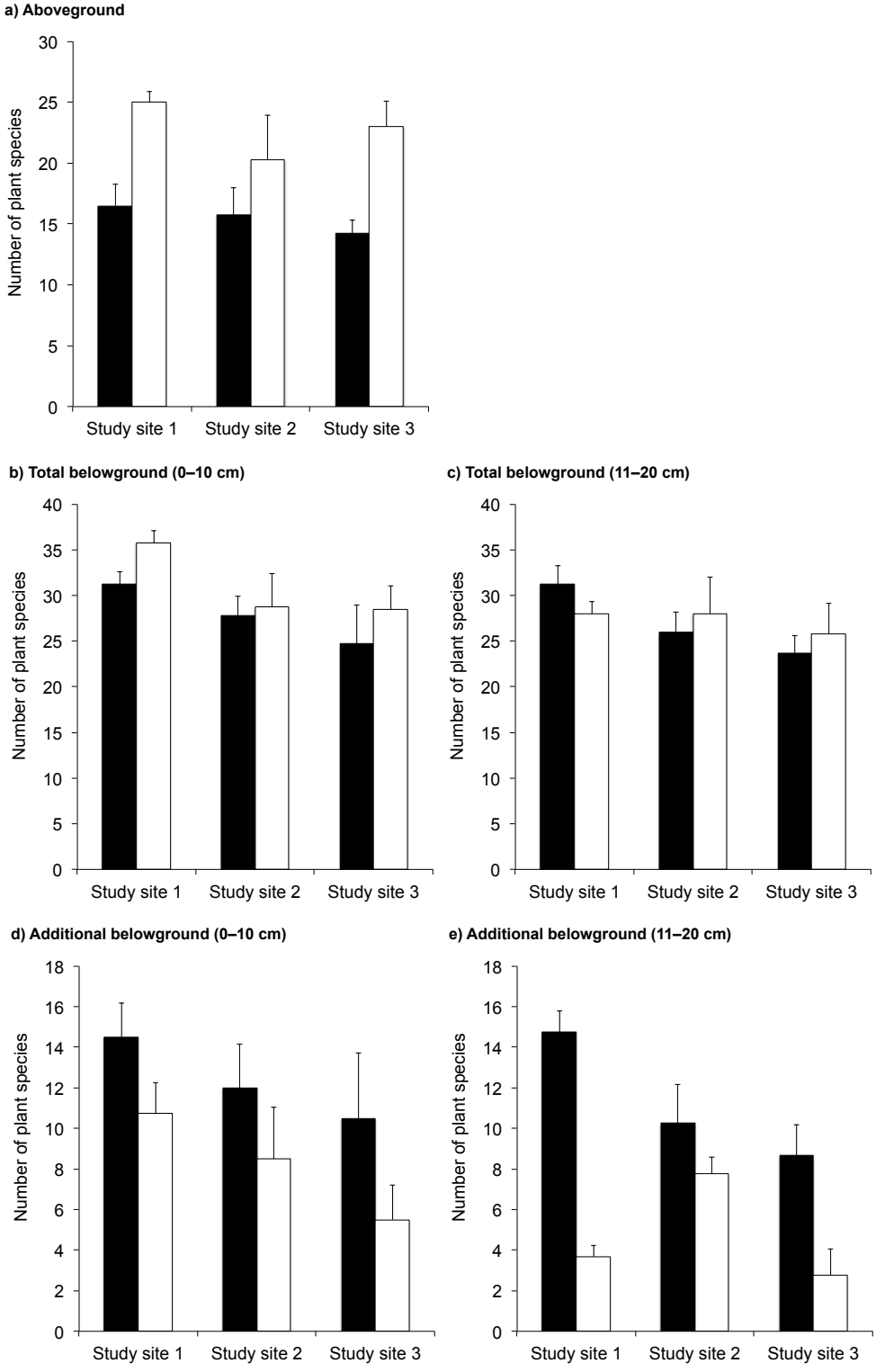
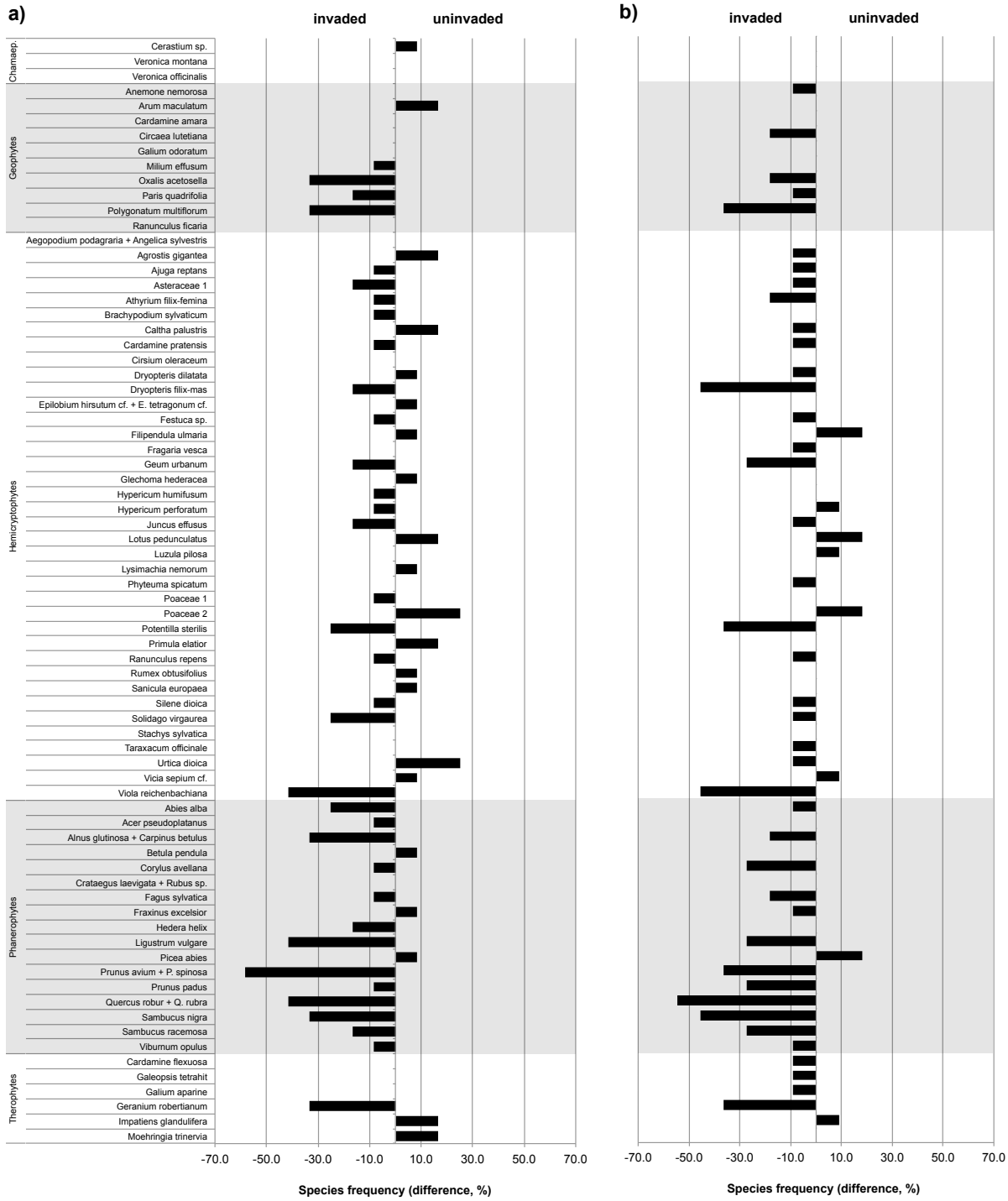


Fig. S4

Differences (expressed in %) between frequencies of occurrence of additional belowground species in invaded and uninvaded plots (**a**: soil depth of 0–10 cm; **b**: 11–20 cm). Plant species are grouped based on their life form (chamaephytes, geophytes, hemicryptophytes, phanerophytes, therophytes), following Landolt et al. (2010)



Reference:

Landolt, E., Bäumler, B., Erhardt, A., Hegg, O., Klötzli, F., Lämmler, W., Nobis, M., Rudmann-Maurer, K., Schweingruber, F.H., Theurillat, J.P., Urmi, E., Vust, M., Wohlgemuth, T., 2010. Flora indicativa - Ecological indicator values and biological attributes of the flora of Switzerland and the Alps. 2nd ed. Haupt, Bern

GENERAL DISCUSSION

Forests cover approximately a third of the European territory and provide important ecosystem services. During the last decades, non-native plants increasingly invaded deciduous and coniferous forests in Central Europe (Nobis 2008; Wagner et al. 2017). Non-native plant species may represent a threat to native biodiversity and ecosystem functioning. The present thesis examines the role of suburban settlements for the spread of non-native plants into surrounding natural forest habitats and investigates how a non-native species, the invasive *Impatiens glandulifera*, affects several components of native ecosystems, with particular focus on belowground diversity. In fact, even if there is increasing evidence that aboveground biodiversity positively affects ecosystem functioning, the relationships between belowground biodiversity and ecosystem functioning and services are rarely studied, and it is not known to which extent invasive plants can disturb these relationships in forests.

In **Chapter I**, I showed that settlements play an important role as a source for the spread of non-native plants into Swiss suburban forests, and that landscape elements such as traffic infrastructure and gardens contribute to their establishment. The higher non-native plant species richness in forests near settlements can be explained by the fact that private gardens are sources of propagules of non-native plants (Sullivan et al. 2005; Marco et al. 2008). Moreover, the high frequency of disturbances from roads, nearby situated settlements and from recreational activities facilitate the establishment of non-native plants in forests (Gavier-Pizarro et al. 2010; McWilliam et al. 2010). Roads adjacent to forests create disturbance and can act as dispersal corridors for non-native species, facilitating plant invasions (Allen et al. 2013; Vakhlamova et al. 2016). The huge variety of ornamental exotic plants cultivated in gardens make them to important sources for non-native plant species, which can escape into the wild (Marco et al. 2008). In fact, most of the non-native species recorded in my study were imported for ornamental purposes. This was also the case for *I. glandulifera*, which was chosen as focal species for further investigations (**Chapters II, III, IV**). *I. glandulifera* was imported into Europe in the middle of the 19th century. By appreciating its high nectar production, beekeepers deliberately introduced *I. glandulifera* into new regions, where it then successfully colonized river banks, moist habitats and, later, forest edges and open forests.

Ruckli et al. (2014a, 2014b, 2016) showed that *I. glandulifera* negatively affects the growth of mycorrhizal fungi and the formation of mycorrhizal symbioses. Based on these findings, I examined the impact of the invasive plant on soil fungal communities in coniferous and deciduous forests, and expected to find a reduced soil fungal diversity in invaded forests (**Chapter II**). Interestingly, I found the opposite result, with a higher fungal diversity and an altered fungal species composition in the presence of *I. glandulifera.* Previous studies showed that invasive plants have the potential to decrease the diversity and

alter the composition of mycorrhizal fungal communities (Mummey and Rillig 2006; Lankau 2011; Zubek et al. 2016). In this respect, it is important to note that the genetic method applied in my study does not allow to determine the function of the different components of the soil fungal community (e.g. mycorrhizal fungi or decomposers). Therefore, I hypothesize that the increase in fungal richness and the change in community composition were presumably a result of a decrease of mycorrhizal fungi, coupled with an increase of saprophytic fungi, which are responsible for the fast decomposition of the large amount of decaying *I. glandulifera* plant biomass in late autumn.

Plant invasions may not only change soil fungal communities, but can also affect the structure and activity of soil bacterial communities (Kourtev et al. 2002; Lorenzo et al. 2013; Qin et al. 2014). Based on the remarkably low leaf litter biomass found in invaded plots, and on the findings of Pattison et al. (2016), who reported an increased bacterial biomass in soils invaded by *I. glandulifera*, I expected to find a higher bacterial activity in the presence of the invasive plant. In contrast, however, bacterial activity was lower in invaded areas than in uninvaded areas in spring, but not in autumn. This finding could be explained by a possible negative influence of the naphthoquinones on soil bacteria, because plant allelopathic compounds often affect soil bacterial communities (Cipollini et al. 2012; Lorenzo et al. 2013) and their concentration in *I. glandulifera* patches is highest in spring (Ruckli et al. 2014b). Paralleling the results of Kourtev et al. (2002), Lorenzo et al. (2013) and Pattison et al. (2016), the invasive plant altered the composition of the soil bacterial community in my study. I also provided evidence that *I. glandulifera* affects deciduous and coniferous forests in a different way: the latter seem to be more sensitive against *I. glandulifera* invasion. The examined coniferous forest stands were dominated by tree species dependent on symbioses with ectomycorrhizal fungi (EMF), and previous field experiments revealed that *I. glandulifera* causes stronger reductions in the degree of mycorrhization in *Fagus sylvatica* (–60%; Ruckli et al. 2016), a species depending on ectomycorrhiza, than in *Acer pseudoplatanus* (–40%; Ruckli et al. 2014a), which depends on arbuscular mycorrhizal fungi (AMF). This makes also sense in the light of a non-strict dependence of *I. glandulifera* on AMF. Some recent studies showed that this invasive plant forms a symbiosis with AMF with a colonization rate varying between 10% and 90% in the non-native range (Štajerová et al. 2009; Tanner et al. 2014; Majewska et al. 2015; Gucwa-Przepióra et al. 2016). As discussed by Tanner and Gange (2013), *I. glandulifera* may not completely eliminate mycorrhizal fungi from invaded stands, but may eliminate some mycorrhizal fungal species, which are more beneficial to native plants.

The study presented in **Chapter III** further examined the effects of *I. glandulifera* on soil fungi, by looking at the spatial extent in which the invasive plant influences mycorrhizal fungi in deciduous forests. I found that *I. glandulifera* strongly reduced the growth of EMF in

invaded patches compared to patches where the invasive plant has been removed. The largest reduction was recorded in the centre of the invaded patches, and hyphal length increased linearly from the centre to the outer part of the patch. In contrast to the findings of my previous study (Chapter II), total soil fungal richness did not differ between invaded and uninvaded areas. This could be caused by the small spatial scale considered (3 m-long transect lines). Nevertheless, I found differences in the composition of the fungal community, paralleling the findings of Chapter II. The reduction in hyphal length recorded in *I. glandulifera* patches is in line with the results of several studies, which showed a decrease in AMF and EMF hyphal length in patches invaded by *Centaurea maculosa* (Mummey and Rillig 2006) and *Alliaria petiolata* (Cantor et al. 2011; Koch et al. 2011). However, it is interesting to note that the recorded reduction in hyphal length caused by the annual *I. glandulifera* (30–70%) is similar to, or even higher, than that reported in other studies investigating the effects of biennial (37%; Cantor et al. 2011) and perennial invasive species (24%; Mummey and Rillig 2006). Extramatrical mycorrhizal mycelia can be involved in so called 'mycorrhizal networks', defined as fungal hyphae that connect the roots of at least two plants and used for the transport of resources within an ecosystem (Newman 1988; van der Heijden and Horton 2009; Simard et al. 2012; Gorzelak et al. 2015). Therefore, the reduced hyphal growth of mycorrhizal fungi found in forest areas invaded by *I. glandulifera* could negatively affect the communication and exchange of resources between trees, reducing also tree fitness and forest regeneration capabilities (van der Heijden and Horton 2009; Simard et al. 2012). Furthermore, a decrease in hyphal biomass would reduce soil stability, and increase the risk of soil erosion (Mummey and Rillig 2006; Rillig and Mummey 2006; Majewska et al. 2018), because fungal hyphae penetrate between soil particles and act as a web to physically retain them. In fact, previous studies showed that *I. glandulifera* increases the risk of soil erosion in riparian habitats (Greenwood and Kuhn, 2014; Greenwood et al., 2018).

In both **Chapters II and III**, I found that soil characteristics, including soil moisture and soil pH, had an influence on the richness and composition of fungal and bacterial communities. Other field experiments demonstrated that *I. glandulifera* can effectively change soil characteristics, for example by increasing soil moisture and soil pH in invaded areas (Ruckli et al. 2013, 2014a). Therefore, I argue that the changes on soil fungal and soil bacterial communities may be an indirect result of the alterations in soil physical and chemical properties induced by the invasion of *I. glandulifera*, in combination with the release of naphthoquinones into the soil.

Finally, the results of **Chapter IV** showed that *I. glandulifera* significantly reduced the root biomass of native plants in invaded forests, probably as a consequence of the allelopathic compounds released by the invasive plant (Schenk et al. 1999), which can negatively affect seedling germination and root elongation (Terzi 2008; Ruckli et al. 2014b). By reducing root

biomass, *I. glandulifera* also reduces soil stability, and this effect is further enforced by the lower hyphal biomass of soil fungi in the presence of the invasive plant, as showed in Chapter III. Moreover, I found that above- and belowground plant communities respond differently to the invasion of *I. glandulifera*. Aboveground plant species richness, assessed through classical vegetation surveys, was lower in invaded than in uninvaded study plots. In contrast, total belowground plant species richness, which was assessed through genetic analyses of mixed root samples, did not differ between invaded and uninvaded plots. Species composition was altered both above- and belowground in invaded forests. Since this is the first study investigating the effects of plant invasions on belowground plant diversity in temperate forests, any comparisons with other studies are not possible in this respect. A closer look to the results, considering the different study sites separately, brought some evidence that the reduction in plant species richness caused by the invasion of *I. glandulifera* was less pronounced in young invaded forest stands than in forests that have been invaded longer time before. This supports the findings of Rusterholz et al. (2017), who reported a time effect in the response of aboveground vegetation to the invasion of *I. glandulifera*, with a delay of about 15 years, and highlights the role of invasion age as a key element to understand the effects of this invasive plant. This may explain the contradicting results found in previous studies investigating the impacts of the invasive species on native plant richness (Maule et al. 2000; Hejda and Pysek 2006; Hulme and Bremner 2006; Hejda et al. 2009; Tanner et al. 2013; Künzi et al. 2015; Diekmann et al. 2016; Čuda et al. 2017; Rusterholz et al. 2017). Unfortunately, most of these studies provided no information on the invasion age of their investigation areas. Furthermore, differences in soil and habitat characteristics may also play a role. In general, the effects of *I. glandulifera* on plant diversity seem to be more pronounced in forests, where the populations are more stable in space and time, than in riparian habitats. In contrast, *I. glandulifera* populations in riparian habitats may be exposed to a high frequency of disturbances by flooding events. Furthermore, in riparian habitats the propagule pressure may be lower than in forests, since part of *I. glandulifera* seeds are brought away by the water course, whereas in forests seeds stay within or near to the patch.

Chapter IV revealed less pronounced impacts of the invasive species on belowground than aboveground plant species richness. I hypothesize that the delay in the effects of *I. glandulifera* on belowground plant species richness may even be longer than that reported by Rusterholz et al. (2017) for the aboveground vegetation. Species might survive for a longer period belowground in form of root or rhizome, and the survival rate of roots could be higher that that of shoots. Many geophytes and woody species occurred belowground but no longer aboveground in plots invaded by *I. glandulifera*. This can be a consequence of both competition for light and allelopathy. In the case of woody species, the low number of seedlings and saplings found aboveground in invaded plots, as a consequence of the negative

effects of *I. glandulifera* on their growth and mycorrhization (Ruckli et al. 2014a, 2014b, 2016), can be problematic in future in terms of regeneration of invaded forests.

Implications and Outlook

The spread of alien plants into forests is expected to increase remarkably in the near future. As discussed in Chapter I, considering the ongoing climate warming, also Northern regions could experience an increased frequency of invasion of non-native plants. In this respect, forests near to settlements are particularly vulnerable, because Chapter I showed that the composition of the surrounding landscape, and in particular gardens and traffic infrastructure, play an important role for the dispersal of non-native plant species. Some of these plants, in turn, can have profound negative effects on native biodiversity and ecosystem functioning. Planting more native plants and shrubs in domestic gardens, and thereby replacing ornamental exotic species would help to reduce the on-going spread of non-native plants into natural habitats.

This thesis also gives new insights on the effects of invasive plants on the belowground biodiversity of temperate forests and assesses potential consequences for ecosystem functioning. The spread of *I. glandulifera* in coniferous and deciduous forests is a serious threat for forest diversity and forest ecosystem functioning. By modifying soil physical and chemical properties, in combination with the release of allelopathic compounds into the soil, *I. glandulifera* is able to alter soil fungal and bacterial communities. This may have consequences on ecosystem processes such as decomposition and nutrient cycling (Bauhus and Khanna 1999; Itoo and Reshi 2013). Moreover, this thesis showed that *I. glandulifera* invasion decreases the growth of mycelia of ectomycorrhizal fungi, which are involved in mycorrhizal networks. This affects negatively the communication and exchange of resources between trees, reducing also tree fitness and ecosystem functions (van der Heijden and Horton 2009; Simard et al. 2012). *I. glandulifera* strongly reduces root biomass of native plants and, combined with the reduction in fungal mycelial growth, this compromises the productivity and stability of forests. Interestingly, this thesis revealed that *I. glandulifera* affects the above- and belowground plant diversity to a different extent. In fact, compared to what happens aboveground, belowground plant diversity seems to respond with a certain delay to the invasion. This provides a buffer period during which it is still possible to restore the previous plant species richness and composition, by extirpating the invasive plant.

As clearly shown by this thesis, the impacts of such an annual invasive plant on forest ecosystems are not negligible. To avoid or reduce such negative effects on forest diversity and forest ecosystem functioning, it is highly recommended to remove the invasive plant in the early stage of the invasion.

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