The invasive plant *Impatiens glandulifera* affects mycorrhizal fungi, plant and invertebrate diversity in deciduous forests

Inauguraldissertartion

zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von

Regina Sirin Lenz-Ruckli
aus Buchrain, LU

Basel, 2015

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
Auf Antrag von

Prof. Dr. Bruno Baur

Prof. Dr. Andres Erhardt

Basel, den 10.12.2013

Prof. Dr. Jörg Schibler
Dekanin/Dekan

Namensnennung-Keine kommerzielle Nutzung-Keine Bearbeitung 3.0 Schweiz
(CC BY-NC-ND 3.0 CH)

Sie dürfen: **Teilen** — den Inhalt kopieren, verbreiten und zugänglich machen

Unter den folgenden Bedingungen:

Namensnennung — Sie müssen den Namen des Autors/Rechteinhabers in der von ihm festgelegten Weise nennen.

Keine kommerzielle Nutzung — Sie dürfen diesen Inhalt nicht für kommerzielle Zwecke nutzen.

Keine Bearbeitung erlaubt — Sie dürfen diesen Inhalt nicht bearbeiten, abwandeln oder in anderer Weise verändern.

Wobei gilt:

- **Verzichtserklärung —** Jede der vorgenannten Bedingungen kann **aufgehoben** werden, sofern Sie die ausdrückliche Einwilligung des Rechteinhabers dazu erhalten.
- **Public Domain (gemeinfreie oder nicht-schützbare Inhalte) —** Soweit das Werk, der Inhalt oder irgendein Teil davon zur Public Domain der jeweiligen Rechtsordnung gehört, wird dieser Status von der Lizenz in keiner Weise berührt.
- **Sonstige Rechte —** Die Lizenz hat keinerlei Einfluss auf die folgenden Rechte:
    - Die Rechte, die jedermann wegen der Schranken des Urheberrechts oder aufgrund gesetzlicher Erlaubnisse zustehen (in einigen Ländern als grundsätzliche Doktrin des **fair use** bekannt);
    - Die **Persönlichkeitsrechte** des Urhebers;
    - Rechte anderer Personen, entweder am Lizenzgegenstand selber oder bezüglich seiner Verwendung, zum Beispiel für **Werbung** oder Privatsphärenschutz.
- **Hinweis —** Bei jeder Nutzung oder Verbreitung müssen Sie anderen alle Lizenzbedingungen mitteilen, die für diesen Inhalt gelten. Am einfachsten ist es, an entsprechender Stelle einen Link auf diese Seite einzubinden.

Quelle: http://creativecommons.org/licenses/by-nc-nd/3.0/ch/ Datum: 12.11.2013

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I am grateful to all my family members, friends and colleagues that supported and encouraged me during my PhD thesis. It is thanks to their help that I could realise my thesis and did not get demoralised and lost my motivation for a fascinating work.

I would like to thank Bruno Baur for giving me the opportunity to carry out this work at the NLU and enabling me to visit conferences and meet there very interesting people that participate my fascination for mycorrhizal symbiosis and invasion biology. I am especially grateful to him for his major help while writing the manuscripts and investing his valuable time during drought periods to help watering the sapling in the field. This project would not have been possible without the help of Hans-Peter Rusterholz who supported me during the whole PhD thesis with usefull advice about statistics and writing and who supported me during fieldwork.

I am very grateful to the endless encouragement from my family. Especially to my parents, Elisabeth and Robert Ruckli, for their constant moral support, and their honest interest in my work. I would like to thank also my sisters Esther and Viola Ruckli, for their help with fieldwork. I am deeply thankful to Armando Lenz for his great moral support and for his patience with me. Further, I am also appreciative for his help with the confusing statistical program R.

Sincere thanks go to my friends. Particularly, I would like to thank Miriam Mühlebach, Deborah Mühlebach, Maya Schefer, Samuel Lüdin, Jonas Küng and Irene Achermann for help with fieldwork and for being fascinated and interested in my work, although they do not understand every detail. Warm thanks go as well to Esther Lischer or help with fieldwork and for encouraging me to be a good teacher.

Further, I would like to thank Katharina Hesse, it was fun to work with her together on the same project and to share the office and the interest about invasion biology.
I would like to thank everyone at NLU who contributed to a good working atmosphere. A particularly thank goes to Samuel Zschokke for the non-scientific discussions, his friendly and humours manner and the help with teaching material. Special thanks go to Eliane Riedener who encouraged me to create a pink poster that won the poster prize; Sijetse Van der Linde who helped me with his suggestions

and comments to improve my manuscripts about mycorrhiza and also for not allowing me to lose the fascination about my work; all my co-authors who scientifically contributed to this thesis and I would like to thank the foresters of Brislachallmet who allowed us to conduct the study in their forest.

SUMMARY

Non-native plant species have the potential to affect an ecosystem by changing species diversity, community structure and interactions among organisms. It is therefore important to understand potential mechanisms that contribute to the success of invasive plants. In the context of my PhD thesis, I examined the effect of the invasive annual herb *I. glandulifera* on mycorrhizal, plant and invertebrate diversity in a deciduous forest near Basel (Switzerland). I also studied the potential mechanism that could contribute to the success of *I. glandulifera*. A controlled field experiment was set up in three forest areas that were affected to a different extend (little, moderatly, high) by a former wind storm. In each area, three different plot types were installed: plots invaded by *I. glandulifera*, plots from which the invasive plants had been manually removed and plots which were no yet colonized by the invasive plant. The results of my thesis demonstrate that *I. glandulifera* affects above and as well as belowground organisms and has the potential to change soil conditions.

Aboveground gastropod richness and abundance were found to be higher in invaded plots than in control plots. The presence of *I. glandulifera* also altered the composition of gastropod species. Belowground fewer types of ectomycorrhiza were found on root systems of *F. sylvatica* saplings. Moreover, in plots with *I. glandulifera* the composition of ectomycorrhiza morphotypes was shifted. Considering mycorrhizal colonisation on fine roots saplings of both tree species, the presence of the invasive plant resulted in a reduction of ectomycorrhizal colonisation of *F. sylvatica* saplings and of arbuscular mycorrhiza colonisation on *A. pseudoplatanus*. Thus, disruption of mycorrhizal symbiosis probably led to a lower biomass and survival rate in saplings of both species grown in invaded plots. Additional results showed that soil moisture, soil pH, available phosphorus and microbial activity were increased in invaded plots.

Increased gastropods abundance and richness could be explained by higher soil moistures and damped soil temperature in invaded plots. However, measured changes in soil conditions did not affect mycorrhizal colonisation and performance of both sapling species. Therefore allelopatic effects of *I. glandulifera* on mycorrhizal symbioses were also examined. Allelopatic compounds are known to contribute to the spread of some invasive plant species and can disrupt mycorrhizal symbiosis. I identified the allelopathic compound 2-methoxy-1,4-naphtoquinone (2-MNQ) in plant organs of *I. glandulifera*, in soil of its proximity and in rain water rinsed from its leaves. Specific bioassays using shoot and root extracts of *I. glandulifera* as well as

synthetic 2-MNQ revealed strong inhibitory effects on mycelium growth of ectomycorrhiza fungi and on the germination of native forest herbs. This finding indicates that the release of 2-MNQ may contribute to the invasion success of *I. glandulifera*.

Health, function and diversity of forest ecosystems are closely linked to the abundance and richness of mycorrhizal fungi. This study demonstrates that the spread *I. glandulifera* into deciduous forest ecosystem resulted in lower mycorrhizal colonisation of saplings. Thus, forest regeneration might be strongly affected by *I. glandulife*ra. Moreover, changes in soil chemistry and soil conditions induced by *I. glandulifera* caused shifts in above- and belowground communities. Therefore ecosystem services and functions of forest habitats can be affected. Removal or stopping the preceding spread of *I. glandulifera* may be an investment for the conservation of native species and may prevent potential losses in timber production.

GENERAL INTRODUCTION

The intentional and unintentional introduction of non-native species is considered as a major threat for the native biodiversity (Pimentel et al. 2005, Lambdon et al. 2008). Non-native plant species have the potential to affect an ecosystem by changing species diversity, community structure and interactions among organisms (Mooney and Hobbs 2000, Kourtev et al. 2002). There is a rapidly increasing number of studies documenting the spread and expansion of non-native species into different habitats, whereas quantitative assessments of their impacts on biodiversity are lagging behind (Pysek et al 2012). Mechanisms and pathways that contribute to the success of invasive species and provide advantages against native species are still poorly studied for many invasive plants. However, a variety of hypotheses have been proposed to explain the invasiveness of non-native plants (e.g. Bloessey and Notzodle 1995; Davis et al. 2000; Bakker and Wilson 2001; Keane and Crawley 2002).

The present doctoral study focuses on following three hypotheses. The first hypothesis considers that disturbance as key factor facilitates the invasion of non-native species into new habitats (Elton 1958, D'Antonio et al. 1999, Hierro et al. 2006, Lockwood et al. 2007). Environmental disturbance is usually assumed to change ecosystem properties providing opportunities for non-native species to invade a new area. The second hypothesis supposes that mechanisms changing the composition and abundance of microbial soil organism increase the success of some invasive plant species (Richardson et al. 2000, Reinhart and Callaway 2006). More specific, invasive plants are able to modify symbiotic interactions between microbial soil organisms and native host plants to their own advantage in the introduced habitats (Rudgers and Orr 2009, Weidenhamer and Callaway 2010). The third hypothesis is the novel weapon hypothesis. It assumes that some invasive plant species produce secondary metabolites that are novel in their non-native ranges. This novelty may provide advantages to the invasive in interactions with naive plants, microbes or generalist herbivores (Callaway and Ridenour, 2004; Inderjit et al. 2011).

In the past decade, the non-native plant *I. glandulifera* increasingly invaded deciduous and coniferous forests disturbed by wind throws and/or intensive forest management (Nobis 2008). *I. glandulifera* 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). It became naturalized and invasive in riparian and disturbed habitats (Hejda and Pysek 2006), and later also in disturbed

forest areas. The presence of *I. glandulifera* causes slight changes in the cover of native plant species and shifts in the species composition in riparian habitats (Maule et al. 2000, Hejda and Pysek 2006). Field studies showed that *I. glandulifera* successfully competes with native plants for pollinators, which can lead to a reduced plant fitness (Chittka and Schurkens 2001). Aqueous shoot extracts of flowering *I. glandulifera* inhibit germination success and root length of *Leucinapis alba* and *Brassica napis* (Vrchotova et al. 2011). Few studies indicated that *I. glandulifera* has allopathic potential contributing to its success (Scharfy et al. 2011). However, the potential impact of *I. glandulifera* on native biodiversity in forest habitats and the general mechanisms that contribute to its invasion success are not known.

I investigated effects of *I. glandulifera* on native forest diversity in a mixed deciduous forest 15 km south of Basel, northern Switzerland. I choose three forest areas that were little, moderately and heavily disturbed by the windstorm “Lothar” in 1999. The invasive plant *I. glandulifera* started to invade the forest, in spring 2000 shortly after the windstorm. Eight years later, I selected homogenous patches of *I. glandulifera* in the three areas. I installed pairs of plots with similar *I. glandulifera* cover. One of the two plots was left invaded by *I. glandulifera*. In the other plot all *I. glandulifera* individuals were removed by hand every spring over 4 years. As an additional control, control plots that were not yet invaded by *I. glandulifera* were selected in close proximity to the experimental plots in each area. This allowed me to control for the slight mechanical disturbance of the soil by removing *I. glandulifera*. To prevent colonisation of *I. glandulifera* in the not yet invaded plots, we removed all invasive plants growing in close proximity to these plots. The additional control plots did not differ in soil characteristics and were colonised by the invasive plant in summer 2012 when the experiments were finished.

FOCUS OF THE STUDY

The **aims of this study** were (1) to examine impacts of *I. glandulifera* on mycorrhizal, plant and invertebrate diversity, and (2) to study the potential mechanisms that could contribute to the invasion success of *I. glandulifera*. In mixed deciduous forests, terrestrial gastropods play an important role as detritivores of leaf litter and senescent plants, and – to a minor extent – as herbivores (Mason, 1970; Wallwork, 1976). In general, terrestrial gastropod communities react sensitive to disturbance and related changes in environmental conditions, including soil pH, soil moisture and calcium carbonate in the upper soil layer as well as the presence/absence of structural elements like woody debris (e.g. Stoll et al., 2009;

Kappes et al., 2009). These factors might be important for shell growth and reproduction in various species (Wäreborn, 1979). Therefore, gastropods are ideal indicators for changes in habitat conditions. **Chapter 1** shows the results of a controlled field experiment aiming to examine whether the invasion of *I. glandulifera* alters gastropod abundance, species richness and the native gastropod community of a mixed deciduous forest which had been disturbed by a wind throw and therefore facilitated the invasion of *I. glandulifera*. The following studies focused on fungal soil diversity and their beneficial association with host plants. Like gastropods diversity, mycorrhizal fungi are indicators of changes in soil conditions, because they interact directly with plants. The roots of many plant species develop associations with particular soil fungi. Symbiosis with mycorrhizal fungi increases both the soil nutrient and water uptake of the host plant, strengthens pathogen resistance and protects the host plant during drought (Smith and Read 2008). The mycorrhizal symbiosis is a key factor determining the diversity of plant communities, their succession dynamics as well as the transport of resources within the ecosystem (Allen 1991, Smith and Read 2009, Johnson et al. 2012). Further, health and function of forest ecosystems are closely linked to abundance and richness of mycorrhizal fungi (Smith and Read, 2008). Therefore, I examined whether the invasion of *I. glandulifera* affected the symbiotic association of young native forest trees and mycorrhizal fungi in a controlled field experiment. **Chapter 2** focused of the abundance of the arbuscular mycorrhiza associated with *Acer pseudoplatanus* saplings, whereas in **Chapter 3** the focus was on the abundance and morphotypes richness of ectomycorrhizal fungi that were associated with *Fagus sylvatica* saplings. Because performance (survival and biomass) of both saplings species were closely linked to abundance of mycorrhizal fungi, I also examined performance of the saplings.

Previous studies showed that some invasive plant species invading natural communities have the potential to disrupt the mycorrhizal symbiosis with host plants (Mummey and Rillig 2006, Wolfe and Klironomas 2008, Rudgers and Orr 2009). In this context allelopathy and the novel weapon hypothesis were frequently discussed. In plant tissues of *I. glandulifera*, naphthoquinones have been identified. Naphthoquinones are known as secondary plant metabolites that could affect soil microbes, fungi and plants in their neighbourhood. In **Chapter 4** I examined the naphthoquinone content in different plant organs of *I. glandulifera*, in buried resin bags of invaded soils and in rainwater dripping from leaves of the invasive plant. The allelopathic potential of aqueous extracts of the invasive plant on native forest plant species and ectomycorrhzal fungi was measured. Therefore, this study contributes to our understanding of how chemical defence can increases success of *I. glandulifera.*

REFERENCES

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge.

Bakker J, Wilson S (2001) Competitive abilities of introduced and native grasses. Plan Ecol 157:117–125

Beerling DJ, Perrins JM (1993) *Impatiens glandulifera* Royle (*Impatiens roylei* Walp.) J Ecol 81:367–382.

Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants – A Hypothesis. J Ecol 83:887–889

Chittka L, Schurkens S (2001) Successful invasion of a floral market - An exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature 411:653–653

Callaway RM, Thelen GC, Barth S, Ramsey PM, Gannon JE (2004) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. Ecology 85:1062–1071

D'Antonio CM, Dudley TL, Mack M (1999) Disturbance and biological invasions: Direct effects and feedbacks. In: Walker LR (Ed.), Ecosystems of disturbed ground. Elsevier, Amsterdam, pp 413–452

Davis MA, Grime JP, Thompson K (2000) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Elton CS (1958) The ecology of invasions by animals and plants. Methuen, London.

Hierro HL, Villarreal D, Eren Ö, Graham JM, Callaway RM (2006) Disturbance facilitates invasions: The effects are stronger abroad than at home. Am Nat 168:144–156

Johnson DF, Martin JW, Cairney G, Anderson IC (2012) The importance of individuals: interspecific diversity of mycorrhizal plants and fungi in ecosystems New Phytol 194:614–628

Kappes H, Jordaens K, Hendrickx F, Maelfait JP, Lens L, Backeljau T (2009) Response of snails and slugs to fragmentation of lowland forests in NW Germany. Landscape Ecol 24: 685–697

Keane RM, Crawley (2002) Exotic plants invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Kourtev PS, Ehrenfeld JG, Haggblom M (2002) Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166

Lambdon PW, Pysek P, Basnou C, Hejda M, Arianoutsou M, Essl F, Jarosik V, Pergl J, Winter M, Anastasiu P, Andriopoulos P, Bazos I, Brundu G, Celesti-Grapow L, Chassot P, Delipetrou P, Josefsson M, Kark S, Klotz S, Kokkoris Y, Kuehn I, Marchante H, Perglova I, Pino J, Vila M, Zikos A, Roy D, Hulme PE (2008) Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs. Preslia 80:101–149

Lookwood JL, Hoopes MP, Marchetti MO (2007) Invasion ecology. Blackwell, Oxford

Mason CR (1970) Snail populations, beech litter production and the role of snails in litter decomposition. Oecologia 5:215–239

Maule H, Andrews M, Watson C, Cherrill AJ (2000) Distribution, biomass and effect on native species of *Impatiens glandulifera* in a deciduous woodland in northeast England. Asp Appl Biol 58:31–38

Mooney HA, Hobbs RJ (2000) Invasive species in a changing world. Island, Washington, DC

Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant Soil 288:81–90

Nobis M (2008) Invasive Neophyten auch im Wald? Wald Holz 8:46–49

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Economy 52:273–288
Pysek P, Vojtech J, Hulme PE, Pergel J, Hejda M, Schaffner U,Vila M (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. Glob Change Biol 18: 1725–1737
Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. New Phytol 170: 445–457
Richardson DM, Allsopp N, D'Antonio CM, Milton SJ, Rejmanek M (2000) Plant invasions - the role of mutualisms. Biol Rev 75:65–93
Rudgers JA, Orr S (2009) Non-native grass alters growth of native tree species via leaf and soil microbes. J. Ecol. 97:247–255
Scharfy D, Funk A, Venterink HO, Güsewell S (2011) Invasive forbs differ functionally from native graminoids, but are similar to native forbs. New Phytol 189:818–828
Smith, SE, Read D (2009) Mycorrhizal symbiosis, 3rd edn. Academic, Amsterdam
Stoll P, Oggier P, Baur B (2009) Population dynamics of six land snail species in experimentally fragmented grassland. J Anim Ecol 78:236–246
Vrchotová N, Šerá B, Krejčová J (2011) Allelopathic activity of extracts from *Impatiens* species. Plant Soil Environ 2:57–60
Wallwork JA (1976) Ecology of Soil Animals. McGraw-Hill, New York
Wäreborn I (1979) Reproduction of two species of land snail in relation to calcium salts in the foerna layer. Malacologia 18:177–180.
Weidenhamer JD, Callaway RM (2010) Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. J Chem Ecol 36:59–69.
Wolfe BE, Rodgers VL, Stinson KA, Pringle A (2008) The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. J Ecol 96:777–783.

CHAPTER 1

Invasion of *Impatiens glandulifera* affects terrestrial gastropods by altering microclimate

Regina Ruckli, Hans-Peter Rusterholz and Bruno Baur

Acta Oecologica 47 (2013) 16–23

Contents lists available at SciVerse ScienceDirect

Acta Oecologica

journal homepage: www.elsevier.com/locate/actoec

Original article

Invasion of *Impatiens glandulifera* affects terrestrial gastropods by altering microclimate

Regina Ruckli*, Hans-Peter Rusterholz, Bruno Baur

Section of Conservation Biology, Department of Environmental Sciences, University of Basel, St. Johanns-Vorstadt 10, CH-4056 Basel, Switzerland

ARTICLE INFO

Article history:
Received 2 October 2012
Accepted 30 October 2012
Available online 17 December 2012

Keywords:
Biodiversity
Deciduous forests
Ecosystem change
Exotic plant
Disturbance
Gastropod community

ABSTRACT

Invasive species can have far-reaching impacts on ecosystems. Invasive plants may be able to change habitat structure and quality. We conducted a field experiment to examine whether the invasive plant *Impatiens glandulifera* affects native terrestrial gastropods. We also evaluated whether the invasive plant alters forest soil characteristics and microclimate which in turn may influence gastropod abundance. We sampled gastropods in plots installed in patches of *I. glandulifera*, in plots in which *I. glandulifera* was regularly removed by hand, and in control plots which were not yet colonized by the invasive plant. The three types of plots were equally distributed over three mixed deciduous forest areas that were slightly, moderately or heavily affected by a wind throw 11 years ago. A total of 33 gastropod species were recorded. Gastropod species richness was not affected by delayed effects of the wind throw, but it was significantly higher in invaded plots than in uninvaded plots. Similarly, gastropod abundance was higher in invaded plots than in the two types of control plots. Canonical correspondence analysis revealed marginally significant shifts of gastropod communities between the three types of plots and indicated that soil moisture, presence of *I. glandulifera* and cover of woody debris affected gastropod species composition. Field measurements showed that soil moisture was higher and daily soil temperature was more damped in patches of *I. glandulifera* than in the native ground vegetation. The changed microclimatic conditions may favour certain gastropod species. In particular, ubiquitous species and species with a high inundation tolerance increased in abundance in plots invaded by *I. glandulifera*. Our field experiment demonstrated that an invasive plant can indirectly affect native organisms by changing soil characteristics and microclimate.

1. Introduction

The intentional and unintentional introduction of non-native species is considered as a major threat for the native biodiversity (Pejchar and Mooney, 2009). There is a rapidly increasing number of studies documenting the spread and expansion of non-native species into different habitats, whereas quantitative assessments of their impacts on biodiversity are lagging behind (Pysek et al., 2012). This is especially true for the potential impact on certain invertebrates groups as well as on detritivores responsible for important ecosystems services and functions.

Impatiens glandulifera, native in the western Himalaya, was introduced as garden ornamental plant to Europa 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 Pysek, 2006). Disturbance is considered as a key factor that facilitates the invasion of non-native species into various habitats (Lookwood et al., 2007). In the last decade, *I. glandulifera* has increasingly invaded deciduous and coniferous forests disturbed by wind throws and/or intensive forest management (Nobis, 2008). *I. glandulifera* is one of the 100 worst alien species in Europe (DAISIE, 2012). Relatively few studies investigated the potential impact of *I. glandulifera* on native plant and animal communities. The presence of *I. glandulifera* causes slight changes in the cover of plant species and shifts in the species composition in riparian habitats (Maule et al., 2000; Hejda and Pysek, 2006). However, compared with other invasive plants (e.g. *Fallopia japonica*, *Solidago gigantea*), the effects of *I. glandulifera* on native plant communities are relatively small (Hejda et al., 2009). Field studies showed that *I. glandulifera* competes successfully with native plants for pollinators, which could lead to a reduced plant fitness (Chittka and Schurkens, 2001). Potential effects of *I. glandulifera* on other invertebrates groups have so far not been investigated.

* Corresponding author. Tel.: +41 61 267 08 51; fax: +41 61 267 08 32.
E-mail address: regina.ruckli@unibas.ch (R. Ruckli).

1146-609X/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.actao.2012.10.011

In temperate forest, terrestrial gastropods play an important role as detritivores of leaf litter and senescent plants, and – to a minor extent – as herbivores (Mason, 1970; Wallwork, 1976). Changes in the gastropod community may affect species with different feeding habits in a different way and thus influence decomposition processes in forests changing this ecosystem function. Gastropods do not feed on fresh plant tissue of *I. glandulifera* but on its senescent leaves and on cotyledons (B. Baur, unpubl. data). The biomass of various plant species is reduced in dense patches of *I. glandulifera*, while the leaf litter layer remains. As most small-sized forest gastropods are living in leaf litter (Kerney et al., 1983) feeding on algae, fungi and senescent plant material (Frömming, 1954), we expected that small snail species occur more frequently in *I. glandulifera* patches than species with large shells. Furthermore, we expected that habitat generalists and species with a high inundation tolerance are less influenced by the invasive plant than habitat specialists with a low inundation tolerance (Bengtsson and Baur, 1993).

Terrestrial gastropod communities react sensitive to disturbance and related changes in environmental conditions, including soil pH, soil moisture and calcium carbonate in the upper soil layer and to the presence/absence of structural elements like woody debris (e.g. Stoll et al., 2009; Kappes et al., 2009). These factors might be important for shell growth and reproduction in various species (Wäreborn, 1979). Because invasion of plants is frequently facilitated by disturbance and gastropods have a limited mobility (Baur and Baur, 1993), we expect that the effect of *I. glandulifera* on gastropod communities is more pronounced in highly disturbed forest areas than in little disturbed areas.

The majority of studies investigating effects of invasive species are field surveys comparing invaded areas with uninvaded ones. Interpretations of their findings should be made with caution because of uncontrolled factors and historical events. Controlled field experiments with a removal of the invasive species are a most valuable and complementary approach. However, such field experiments are still rare in invasion ecology (Hulme and Bremner, 2006; Lopezaraiza-Mikel et al., 2007; Nienhuis et al., 2009).

We designed a controlled field experiment to examine whether the invasion of *I. glandulifera* alters the native gastropod community of a mixed deciduous forest which had been disturbed by a wind throw. We set up experimental plots in patches of *I. glandulifera*, in patches in which the invasive plant was regularly removed by hand, and in plots in neighbouring areas not yet colonized by the plant. The three plot types were equally distributed over three forest areas that were slightly, moderately and heavily affected by a wind throw 11 years ago, representing a natural gradient of disturbance. We examined the terrestrial gastropod communities in the different plots and compared the life-history traits of the occurring species to answer the following questions: (1) Does the invasion of *I. glandulifera* affect the species richness and abundance of terrestrial gastropods? (2) Does the presence of *I. glandulifera* alter the species composition of the gastropod community? and (3) Are gastropods species with particular life-history traits (shell size, inundation tolerance and habitat specifity) or a combination of these traits more severely affected by the invasion of *I. glandulifera* than species with other traits?

2. Material and methods

2.1. Study system and field experiment

The experiment was carried out in a mixed deciduous forest dominated by *Fagus sylvatica* 15 km south of Basel, northern Switzerland (47°43′ N, 7°55′ E). In this region the annual temperature averages 9.6 °C and the annual precipitation is 1021 mm (Meteo Swiss, 2009). We selected study plots in three areas (each measuring 50 m × 180 m), which were differently affected by the windstorm Lothar in 1999. We estimated the disturbance intensity of the storm by assessing the canopy closure of the remaining forest trees in the three areas 8 years after the event. We defined the first area with a canopy closure of 80% as slightly disturbed, the second with a canopy cover of 50% as moderately disturbed, and the third with a canopy closure of 2.5% as heavily disturbed (Table 1). The three areas were situated within 1 km^2. *I. glandulifera* started to invade the forest shortly after the storm.

In spring 2008, we selected six homogenous patches of *I. glandulifera* in each area and installed two 5 m × 5 m plots with similar *I. glandulifera* cover in each patch adjacent to each other. In one of the two plots we removed all *I. glandulifera* individuals by hand (hereafter referred to as removed) every spring in the years 2008–2010. The other plot was left invaded by *I. glandulifera* (hereafter referred to as invaded). As an additional control, we selected six 5 m × 5 m control plots that were not yet invaded by *I. glandulifera* in close proximity (7–20 m) to the experimental plots in each area (referred to as uninvaded). To prevent so far uninvaded plots from the colonisation of *I. glandulifera*, we removed all individuals occurring close to these plots in spring 2010. In 2012, two years after the present study, most of formerly uninvaded plots were colonized by *I. glandulifera.* In our experiment, the uninvaded plots allowed to examine the potential effect of manual removal of *I. glandulifera* in that treatment. Furthermore, the uninvaded plots allowed an assessment of the gastropods community in disturbed, but not yet invaded forest areas. Finally, the uninvaded plots allowed to check whether the invasion of *I. glandulifera* changed soil characteristics. It is assumed that the different plots situated in

Table 1
Environmental characteristics of the three study areas that were differently disturbed by a windstorm. *P* and *F*-values resulting from ANCOVA indicating differences between study areas are shown.

	Intensity of disturbance			ANCOVA	
	Low	Moderate	High	*F*	*P*
Canopy closure (%)[a]	80 (50–80)	50 (50–70)	2.5 (0–10)	–	–
Forest vegetation type[b]	Galio oderati-Fagetum luzuletosum	Luzulo sylvaticae-Fagetum typicum	Galio oderati-Fagetum luzuletosum	–	–
Soil type[c]	Eutric haplic luvisol	Haplic luvisol	Gleyic cambisol	–	–
Density of *I. glandulifera* (m^{-2})[d]	203.3 ± 34.0	179.2 ± 14.6	215.3 ± 25.6	0.20	0.659
Biomass of *I. glandulifera* (gm^{-2})[d]	237.9 ± 58.1	243.7 ± 54.9	118.9 ± 28.8	3.46	0.085

Mean values ± SE, $n = 18$ plots for each intensity of disturbance.
[a] Median and range.
[b] Burnand and Hasspacher (1999).
[c] Walthert et al. (2004).
[d] $n = 6$.

close proximity did not differ in soil characteristics prior to the invasion of *I. glandulifera*. Thus, the experimental set-up consisted of 54 plots (18 removed, 18 invaded and 18 uninvaded) equally distributed over the three areas, which were differently affected by the storm.

2.2. Data collection

Two methods were applied to assess the effect of *I. glandulifera* on species richness and relative abundance of terrestrial gastropods. First, we visually searched for living gastropods and empty shells in each plot for 15 min. The visual search was restricted to the morning (between 08:00 and 11:00 a.m.) to minimize variation in finding success due to daytime-related gastropod activity. Second, we collected soil and litter samples including dead plant material at randomly chosen spots in each sampling plot (0.5 L soil per plot). The soil samples were dried at 60 °C for 24 h. Then, samples were put through sieves with decreasing mesh sizes of 2, 1 and 0.2 mm and later examined under a binocular microscope. Gastropod shells were sorted out of the samples and identified according to Kerney et al. (1983). To minimize edge effects, this survey was restricted to a 2 m × 2 m subplot installed in the centre of each plot. Gastropod surveys were conducted from 3 to 14 June and repeated from 9 to 20 September 2010, resulting in a total searching time of 30 min and a soil and litter sample of 1 L per plot.

We measured the soil temperature at a depth of 5 cm in nine plots (three invaded, three removed and three uninvaded equally distributed over the three areas) during the periods of gastropod sampling using Tinytalk temperature loggers (Gemini Data Loggers, Chichester, UK). Temperature was recordered at intervals of 3 h. We estimated the cover of the ground vegetation (excluding *I. glandulifera*) and the cover of woody debris in each 2 m × 2 m subplot using the Domin scale (Mueller-Dombois and Ellenberg, 2002) at the end of September 2010. In invaded plots, we counted the number of *I. glandulifera* individuals. We also assessed the biomass (dry weight including roots) of *I. glandulifera* by removing all plants from subplots measuring 0.25 m^2 at the end of September 2010.

We removed the leaf litter layer and collected five soil samples to a depth of 5 cm using a metal cylinder of 5 cm diameter (soil volume 100 cm^3). The soil samples were taken at distances of 20 cm along a line in each plot. The five soil samples collected in each plot were pooled and mixed, resulting in a total of 54 samples. We determined soil moisture (%) using the fresh weight to dry weight ratio. Soil pH was assessed in distilled water (1:2.5 soil:water; Grimshaw, 1989). We determined total soil organic matter content (SOM) as loss-on-ignition of oven-dried soil at 700 °C for 23 h (Grimshaw, 1989). Calcium carbonate content ($CaCO_3$, in %) was assessed by adding hydrochloric acid (10 ml HCL) and back titration with sodium hydroxide (NaOH; Nelson and Sommers, 1996).

2.3. Gastropod characteristics

To examine whether gastropod species with different habitat specificity were differentially affected by the presence of *I. glandulifera*, we assigned all snail species to one of the following categories: open-land (species exclusively occurring in open habitat), forest (species mainly found in forests) or ubiquitous species (species found in different types of habitats) following Kerney et al. (1983) and Falkner et al. (2001) (see Appendix 1). Similarly, species-specific data on inundation tolerance (low, moderate or high), adult shell size (small: shell height or breadth < 5.0 mm; large: shell high or breadth ≥ 5.0 mm), age at sexual maturity (1: <1 year; 2: 1 year; 3: >1 year) and longevity (<2 years or ≥2 years) were extracted from Kerney et al. (1983) and Falkner et al. (2001). Data on egg size and clutch size were obtained from Bengtsson and Baur (1993) and B. Baur (unpubl. data).

2.4. Statistical analyses

We used R statistic (R Development Core Team, 2010; version 2.12.1) for all analyses. Preliminary analyses showed that the gastropod data from the two sampling periods (June/September) did not differ. We therefore combined the data for further analyses. To examine the effect of *I. glandulifera* on environmental characteristics (soil moisture, soil pH, soil organic matter, calcium carbonate content, ground plant cover and cover of woody debris) we used analyses of covariance (ANCOVA) with treatment (invaded, removed, uninvaded) as factor and disturbance (expressed as canopy cover) as cofactor. To examine the effect of *I. glandulifera* on gastropod species richness and diversity, we used analyses of covariance (ANCOVA) with plot and treatment as factors and disturbance, soil moisture, soil pH, soil organic matter, concentration of calcium carbonate, ground plant cover and cover of woody debris as cofactors (Table 3). Tukey HSD tests were used to examine differences between treatments. We used the same ANCOVA-model to examine whether the invasion of *I. glandulifera* influences the abundance of gastropods. The statistical models were stepwise reduced as recommended by Crawley (2007). All interactions were non-significant. If necessary data were square root-transformed to obtain normally distributed residuals and homogeneous group variances. Individual-based rarefaction curves were calculated using the package vegan (R Development Core Team, 2010; version 2.12.1).

Canonical correspondence analysis (CCA) was applied to examine gastropod community composition in relation to environmental variation using CANOCO version 4.5 (ter Braak and Smilauer, 2002). To identify environmental variation related to different treatments, presence or absence of *I. glandulifera* and the cover of ground vegetation and soil characteristics were considered in the CCA. We used the entire dataset including all gastropod species recorded in the CCA with biplot scaling. Monte-Carlo

Table 2
Environmental characteristics of the three types of plots (invaded, invasive plant removed, uninvaded). *P* and *F*-values resulting from ANCOVA's indicating differences between the different treatments and disturbance intensities (corresponding figures are shown in Appendix B (Fig. B1)).

	Treatments			ANCOVA			
	Invaded	Removed	Uninvaded	Treatment		Disturbance	
				F	*P*	*F*	*P*
Soil moisture (%)	31.6 ± 0.9	25.7 ± 1.1	21.2 ± 0.8	9.38	**<0.0001**	70.43	**<0.0001**
Ground plant cover (%)	83 ± 3	68 ± 5	73 ± 4	1.17	0.316	4.12	**0.047**
Soil pH	4.7 ± 0.05	4.6 ± 0.07	4.7 ± 0.04	0.86	0.427	0.06	0.802
Total soil organic matter (%)	18.0 ± 1.0	16.9 ± 1.1	12.8 ± 0.6	1.08	0.349	15.31	**<0.0001**
$CaCO_3$ (%)	3.6 ± 0.02	3.7 ± 0.03	3.6 ± 0.03	1.04	0.360	0.67	0.417
Woody debris cover (%)	35.8 ± 1.4	10.6 ± 1.4	13.1 ± 1.7	14.37	**<0.0001**	62.24	**<0.0001**

Mean values ± SE, $n = 18$ plots for each intensity of disturbance.
Significant *P*-values (<0.05) are indicated in bold.

Table 3
Summary of ANCOVA's testing the effects of disturbance (area), treatment (invaded, removed and uninvaded), plot, cover of plant and woody debris and different soil characteristics on species richness, abundance and diversity of gastropods.

	Richness			Abundance			Fisher's alpha		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Intensity of disturbance	1,32	0.13	0.72	1,29	15.89	**<0.001**	1,33	12.26	**0.001**
Treatment	2,32	7.42	**0.002**	2,29	3.32	**0.045**	2,33	5.11	**0.011**
Plot [Treatment]	15,32	0.99	0.48	15,29	1.51	0.16	15,33	1.13	0.37
Soil pH	1,32	10.36	**0.003**	1,29	2.23	0.14	1,33	6.57	**0.015**
Soil moisture (%)	1,32	2.98	0.09	–	–	–	–	–	–
Woody debris cover (%)	–	–	–	1,29	5.02	**0.032**	–	–	–
Plant cover (%)	–	–	–	1,29	1.38	0.25	–	–	–
Soil $CaCO_3$ (%)	–	–	–	1,29	1.28	0.27	–	–	–
Total soil organic matter (%)	–	–	–	–	–	–	1,33	2.30	0.14

"– " indicates that the variables were excluded from the model.
Significant *P*-values (<0.05) are indicated in bold.

permutation tests (499 permutations) were conducted to evaluate the significance of the environmental variables. The obtained additional (lambda A) and absolute effects (lambda B) for each environmental variable estimated the likelihood that the environmental variables affected the composition of gastropods species. We applied an one-way ANOVA to examine whether the gastropod communities sampled in plots of three different treatments could be separated by scores obtained from the CCA analysis.

Correlation analysis showed that shell size, age at sexual maturity, longevity, egg size and clutch size were all intercorrelated (in all cases, $P < 0.001$). Consequently, shell size can be considered as surrogate for the other life-history traits. We applied contingency table tests to evaluate differences in the proportion of gastropod species and individuals showing different habitat specificity, inundation tolerance and different life-history traits including age at sexual maturity and longevity between the treatments (invaded, removed, uninvaded).

3. Results

3.1. Environmental characteristics

Soil moisture was 12–18% higher in plots invaded by *I. glandulifera* and in plots in which the invasive plant was removed than in uninvaded plots (Table 2). Similarly, the cover of woody debris was higher in plots invaded by *I. glandulifera* and in plots in which the invasive plant was removed than in uninvaded plots (Table 2). Soil temperature in the sampling periods in June and September 2010 was 2–5 °C lower in invaded plots than both in plots in which the plant was removed and in uninvaded plots (Fig. 1). In contrast, the cover of ground vegetation, soil pH, soil organic matter and concentration of calcium carbonate did not differ among invaded plots, plots in which the invasive plant has been removed and uninvaded plots (in all cases, $P > 0.3$, Table 2). There was no interaction between treatment and intensity of disturbance in any of the habitat characteristics examined.

3.2. Gastropod species richness, abundance and diversity

A total of 33 gastropod species were recorded, 31 of them in invaded plots, 25 in removed and 24 in uninvaded plots (Appendix A). Gastropod species richness was not affected by canopy closure (intensity of disturbance; Table 3). However, gastropod species richness was significantly higher in invaded plots than in uninvaded plots (Table 3, Fig. 2). Furthermore, independent of treatment, gastropod richness increased with increasing soil pH (Table 3). Rarefaction curves indicate that the higher species richness in invaded plots resulted from the higher number of gastropods sampled in this plot type.

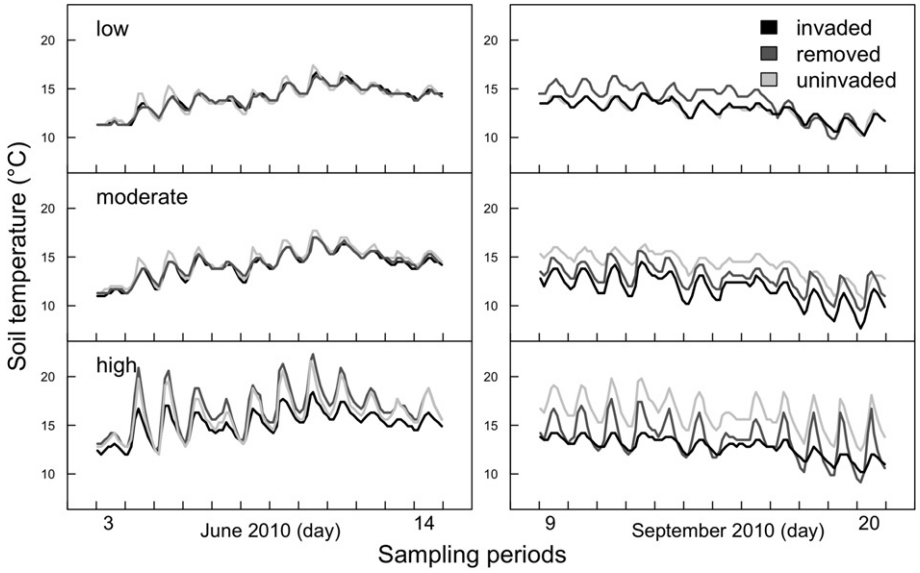

Fig. 1. Soil temperature during the two sampling periods in invaded plots, in plots with removed *I. glandulifera*, and in uninvaded plots in slightly, moderately, and highly disturbed forest areas. Soil temperature was recorded at intervals of 3 h. Mean values of three data loggers are shown in each case.

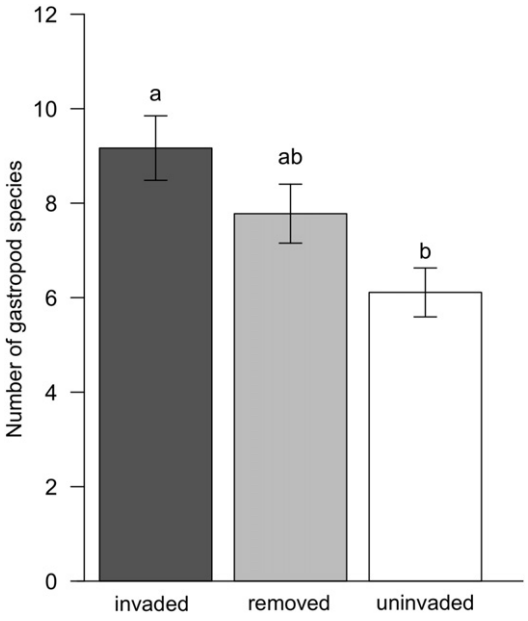

Fig. 2. Gastropod species richness (mean ± SE) in plots invaded by *I. glandulifera*, in plots in which *I. glandulifera* had been removed and in uninvaded plots. Data were averaged across the treatments ($n = 18$ in each case, Table 2).

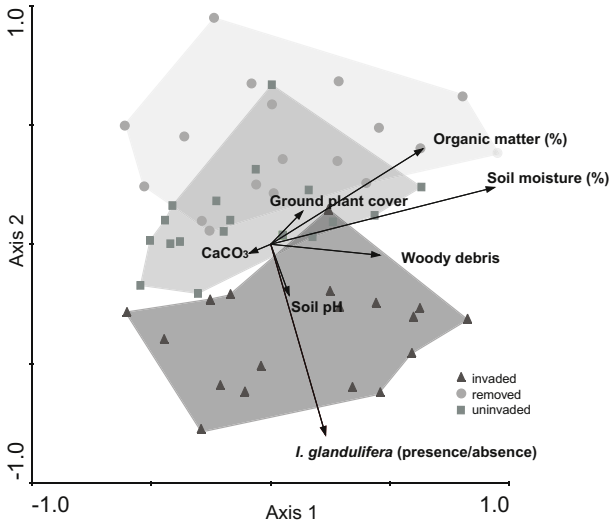

Fig. 4. First and second axis of CCA ordination (bi-plot-scaling) for the plots invaded by *I. glandulifera* (filled triangles), plots in which *I. glandulifera* has been removed (filled squares) and uninvaded plots (filled circles). Convex hulls, connecting the most outward lying sites, were drawn to visualize changes in the gastropod communities.

A total of 1178 gastropod individuals were recorded, 462 (39.2%) of them in invaded plots, 360 (30.6%) in removed plots and 356 (30.2%) in uninvaded plots (Appendix A). The number of gastropods sampled decreased significantly with increasing intensity of disturbance (Fig. 3, Table 3). Furthermore, in the highly disturbed area gastropod abundance was significantly higher in invaded plots than in both uninvaded plots and plots from which *I. glandulifera* had been removed (Fig. 3, Table 3). The number of gastropod individuals sampled was also negatively influenced by the cover of woody debris (Table 3).

Gastropod diversity (Fisher's alpha) was affected by both the intensity of disturbance and the presence of *I. glandulifera* (Table 3). Gastropod diversity increased with increasing level of disturbance (mean ± SE; low: 3.87. ± 0.51; moderate: 5.00 ± 0.53; high: 6.74 ± 0.02). Furthermore, gastropod diversity was significantly higher in invaded plots (6.10 ± 0.70) than in uninvaded plots (3.68 ± 0.40) (Tukey HSD-test; $P < 0.05$; Table 2). However, the diversity in invaded plots did not differ from that in plots in which *I. glandulifera* had been removed (5.83 ± 0.84).

3.3. Community composition

The first three axes of the CCA explained 17.1% of the gastropod species variation, and 80.7% of the species–environment relationship. The multivariate analysis showed a slight separation of the gastropod communities in invaded plots and in plots with removed *I. glandulifera* (axis 2: $F_{1,34} = 3.13$, $P = 0.085$) and between the communities in invaded and uninvaded plots (axis 2: $F_{1,34} = 3.26$, $P = 0.079$; Fig. 4). However, the CCA also revealed that soil moisture, presence or absence of *I. glandulifera* and cover of woody debris affected the gastropod communities (Table 4, Fig. 4). In contrast, the cover of ground vegetation, concentration of calcium carbonate, soil pH and total organic matter in the soil did not influence the gastropod communities (Table 4).

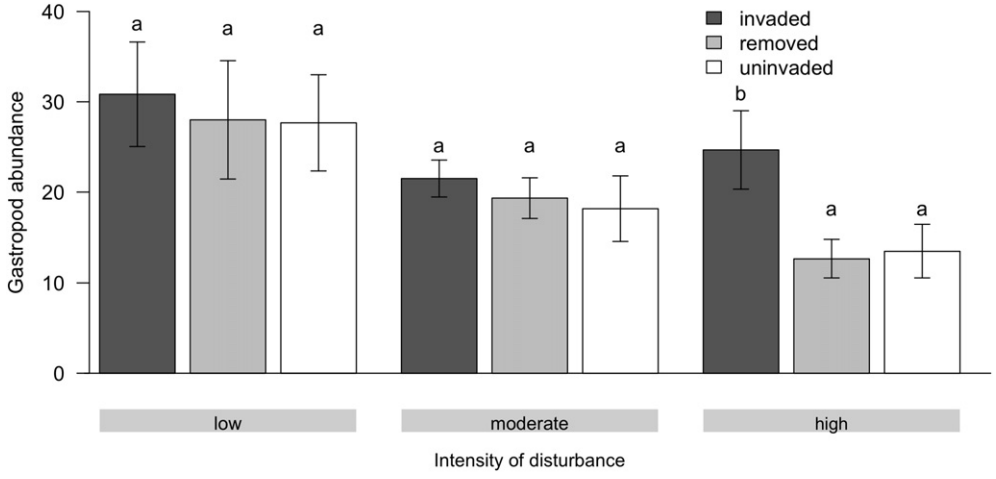

Fig. 3. Abundance of gastropods (mean ± SE) in plots invaded *by I. glandulifera*, in plots in which *I. glandulifera* had been removed, and in uninvaded plots in slightly, moderately and highly disturbed forest areas. Different letters indicate significant differences between the different types of plots within area (Tukey HSD, $P < 0.05$).

Table 4
Results of the canonical correspondence analysis (CCA) showing the variance values of absolute and additional effects explained by environmental variables.

Absolute effects		Additional effects		
Variable	Lambda I	Lambda A	*F*	*P*
Soil moisture (%)	0.17	0.17	4.92	**0.002**
I. glandulifera (presence/absence)	0.07	0.08	2.05	**0.004**
Woody debris cover (%)	0.07	0.06	2.00	**0.008**
Plant cover (%)	0.04	0.05	1.28	0.17
Soil $CaCO_3$ (%)	0.03	0.03	0.85	0.62
Soil pH	0.03	0.02	0.78	0.74
Total soil organic matter (%)	0.10	0.02	0.44	0.99

Environmental variables are listened in the order of automatic forward selection by CANOCO.
Significant *P*-values (<0.05) are indicated in bold.

3.4. Gastropod characteristics

The three types of plots did not differ in frequencies of forest (48%), open-land (12%) and ubiquitous (40%) gastropod species ($X^2 = 0.17$, df = 4, $P = 0.99$). Furthermore, the three types of plots harboured similar frequencies of gastropod species with low, moderate and high inundation tolerance ($X^2 = 3.24$, df = 4, $P = 0.52$). However, the presence of *I. glandulifera* altered the frequencies of gastropod individuals with different habitat specificity ($X^2 = 12.14$, df = 4, $P = 0.016$). In invaded plots, more open-land (7.5%) and ubiquitous individuals (33.1%) were recorded than both in plots with removed *I. glandulifera* and in uninvaded plots (combined: 4.5% open-land and 26.9% ubiquitous gastropod individuals). Furthermore, invaded plots and plots with removed *I. glandulifera* had larger proportions of gastropod individuals with high inundation tolerance (10.3% and 11.6%) than uninvaded plots (3.8%; $X^2 = 37.63$, df = 4, $P < 0.0001$). Finally, the three types of plots did not differ in the size distribution of gastropod individuals ($X^2 = 2.03$, df = 2, $P = 0.36$).

4. Discussion

Our field experiment showed an increase in gastropod species richness in response to *I. glandulifera* invasion independent of the degree of forest disturbance. The presence of *I. glandulifera* caused a slight shift in the gastropod community. Furthermore, in the highly disturbed forest area the abundance of gastropods was higher in invaded plots than in uninvaded plots.

The diversity of vascular plants plays a key role for the species richness and abundance of terrestrial gastropods in a variety of habitats (Horsak and Hajek, 2003; Saetersdal et al., 2004; Horsak et al., 2010). However, there are several ecosystems including forests in which gastropod diversity and abundance are not related to the diversity of vegetation (Bishop, 1980; Baur et al., 1996; Cremene et al., 2005). In our field experiment, species richness and composition of vascular plants were not affected by *I. glandulifera* (Küng, 2010). This result is in line with findings of Hulme and Bremner (2006) and Hejda et al. (2009) showing that the invasion of *I. glandulifera* caused only slight changes in the native vegetation. Therefore, food resources and microhabitat heterogeneity for terrestrial gastropods may not be significantly reduced by the invasion of *I. glandulifera*. It is even possible that the invasive plant provides an additional microhabitat or a structural element that is favourable for certain terrestrial gastropod species. This may result in higher species richness and abundance of gastropods, especially in highly disturbed areas. In contrast, the invasive Japanese knotweed *F. japonica* causes a significant reduction in species richness and abundance of gastropods (Kappes et al., 2007; Gerber et al., 2008; Stoll et al., 2012). With its dominant stands the knotweed reduces the native plant species richness, and thus alters the food resources and structural habitat heterogeneity for terrestrial gastropods (Boycott, 1934; Labaune and Magnin, 2001).

Besides vascular plant diversity, the calcium carbonate content of the soil, temperature, humidity and different types of disturbances are known to influence the richness and abundance of gastropods (e.g. Denslow, 1980; Wäreborn, 1992; Johannessen and Solhøy, 2001; Boschi and Baur, 2008). Invasive plants may alter the soil moisture, soil conditions, nutrient cycling and the microclimate (Ehrenfeld et al., 2005; Reinhart and Callaway, 2006; Munoz Valles et al., 2011). In our study, soil moisture was higher in invaded plots and plots in which *I. glandulifera* was removed than in uninvaded plots. In general, the richness and abundance of gastropods are positively related to soil moisture content (Wäreborn, 1992; Martin and Sommer, 2004; Horsak and Cernohorsky, 2008). Thus, it is not surprising that we found more gastropod species in patches, which retained soil moisture. High soil moisture results in a higher evaporation, which in turn reduces soil temperature (as measured in the present study). The soil remained moist in the plots in which *I. glandulifera* had been repeatedly removed during the growing season. It is possible that *I. glandulifera* prefers to invade forest areas with high soil moisture. However, this seems not to be the case in our field experiment. Most of the not yet invaded plots in 2010 were two years later colonized by the invasive plant. Our experimental removal plots may constitute small gaps in the *I. glandulifera* patches. The dense surrounding stands consisting of up to 2.5 m tall invasive plants may shade the small gaps (5 m × 5 m) and thus influence the soil moisture of these plots. Indeed, after the senescence of *I. glandulifera* in December 2010, soil moisture did no longer differ between the plots of the different treatments (H.-P. Rusterholz, unpubl. data).

We recorded an increase in gastropod abundance in invaded and highly disturbed plots. In the highly disturbed area, fluctuations of daily air and soil temperature were dampened in invaded plots compared to the other plot types (Fig. 1). The canopy is still more open in the highly disturbed forest area than in the other two forest areas, even 11 years after the wind throw. As a result of the open canopy, air and soil temperature and evaporation rate are increased (Denslow, 1980). Under these conditions, dense stands of *I. glandulifera* are providing a favourable microclimate for terrestrial gastropods with higher soil moisture and lower temperature fluctuations (Boag, 1990; Hawkins et al., 1998). Thus, several gastropod species might be attracted by the moister conditions in areas invaded by *I. glandulifera*, resulting in higher gastropod richness and abundance.

Beside soil moisture and temperature, *I. glandulifera* may attract certain gastropod species as food plant and thereby increase species richness and abundance. In late spring, grazing damage by gastropods has been observed on cotyledons of *I. glandulifera* (B. Baur, personal observation). In contrast, gastropods did rarely feed on primary and secondary leaves of the invasive plant, most probably due to their specific chemical secondary compounds (R. Ruckli, unpubl. data). In controlled food-choice tests, gastropods did not feed on fresh leaves but consumed senescent leaves of *I. glandulifera* (B. Baur, unpubl. data).

The repeated removal of individuals of *I. glandulifera* in one treatment may not affect the species richness and abundance of terrestrial gastropods. Plant removal began 3 years before the gastropod community was examined. Since then, the number of invasive plants removed decreased because less seeds occurred in the seed bank.

In general, disturbances alter gastropods communities (e.g. Gerber et al., 2008; Stoll et al., 2009; Bros et al., 2011). Gastropod species differ considerably in sensitivity to disturbance (Boschi and

Baur, 2007a, b). In deciduous forest, species with a high forest habitat specificity are sensitive to disturbance (Kappes et al., 2009). Our study also revealed that the proportion of gastropod individuals with different habitat specificity was altered in patches of *I. glandulifera*. Invaded plots harboured a lower proportion of forest individuals than control plots. Depending on the type and extent of disturbance, the proportion of forest individuals is highly variable in deciduous forests. The percentages of forest gastropod individuals varied from 33 to 45% in disturbed beech forests in Germany (Kappes et al., 2009), from 25 to 42% in natural deciduous forest in Eastern Europe (Cameron and Pokryszko, 2004), and from 19 to 69% in temperate-humid forests in Germany (Martin and Sommer, 2004). In the present study, the percentage of forest gastropod individuals ranged from 58 to 69%. The lower abundance of forest individuals in invaded plots and the slight shift in the composition of communities indicate that the invasion of *I. glandulifera* alters gastropod communities.

Our findings demonstrate the potential of *I. glandulifera* to induce functional changes in forest ecosystems by increasing soil-moisture and dampening daily soil temperature fluctuations in highly disturbed areas. In this way, the presence of *I. glandulifera* provides favourable environmental conditions for certain gastropod species during summer.

Acknowledgements

We thank A. Baur and two anonymous reviewers for comments on the manuscript, J. Piza for field assistance, and T. Meier for help in gastropod species determination. Financial support was received from the Velux Stiftung Zürich.

Appendix A and B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.actao.2012.10.011.

References

Baur, A., Baur, B., 1993. Daily movement patterns and dispersal in the land snail *Arianta arbustorum*. Malacologia 35, 89–98.
Baur, B., Joshi, J., Schmid, B., Hänggi, A., Borcard, D., Stary, J., Pedroli-Christen, A., Thommen, G.H., Luka, H., Rusterholz, H.-P., Oggier, P., Ledergerber, S., Erhardt, A., 1996. Variation in species richness of plants and diverse groups of invertebrates in three calcareous grasslands of the Swiss Jura mountains. Rev. Suisse Zool. 103, 801–833.
Beerling, D.J., Perrins, J.M., 1993. *Impatiens glandulifera* Royle (*Impatiens roylei* Walp). J. Ecol. 81, 367–382.
Bengtsson, J., Baur, B., 1993. Do pioneers have *r*-selected traits – life-history patterns among colonizing terrestrial gastropods. Oecologia 94, 17–22.
Bishop, M.J., 1980. The Mollusca of acid woodland in the Italian province of Novara. J. Conchol 30, 181–188.
Boag, D.A., 1990. On the effectiveness of artificial shelters in the study of population attributes of small terrestrial gastropods. Can. J. Zool. 63, 1089–1095.
Boschi, C., Baur, B., 2007a. Effects of management intensity on land snails in Swiss nutrient-poor pastures. Agr. Ecosyst. Environ. 120, 243–249.
Boschi, C., Baur, B., 2007b. The effect of horse, cattle and sheep grazing on the diversity and abundance of land snails in nutrient-poor calcareous grasslands. Basic Appl. Ecol. 8, 55–65.
Boschi, C., Baur, B., 2008. Past pasture management affects the land snail diversity in nutrient-poor calcareous grasslands. Basic Appl. Ecol. 9, 752–761.
Boycott, A.E., 1934. The habitats of land mollusca in Britain. J. Ecol. 22, 1–38.
Bros, V., Moreno-Rueda, G., Santos, X., 2011. Does postfire management affect the recovery of Mediterranean communities? The case study of terrestrial gastropods. For. Ecol. Manage. 261, 611–619.
Burnand, J., Hasspacher, B., 1999. Waldstandorte beider Basel. Quellen und Forschungen zur Geschichte und Landeskunde des Kantons Basel-Landschaft, Band 72. Verlag des Kantons Basel-Landschaft, Liestal.
Cameron, R.A.D., Pokryszko, B.M., 2004. Land mollusc faunas of Bialowieza forest (Poland), and the character and survival of forest faunas in the North European Plain. J. Mollus. Stud. 70, 149–164.
Chittka, L., Schurkens, S., 2001. Successful invasion of a floral market – An exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature 411, 653.
Crawley, M.J., 2007. The R Book. Wiley, Chichester, England.
Cremene, C., Groza, G., Rakosy, L., Schileyko, A.A., Baur, A., Erhardt, A., 2005. Alterations of stepp-like grasslands in eastern Europe: a threat to regional biodiversity hotspots. Conserv. Biol. 19, 1606–1618.
DAISIE European Invasive Alien Species Gateway, 2012. *Impatiens glandulifera* (accessed 01.04.12.). http://www.europe-aliens.org/speciesFactsheet.do?speciesId=17367.
Denslow, J.S., 1980. Patterns of plant species diversity during succession under different disturbance regimes. Oecologia 46, 18–21.
Ehrenfeld, J.G., Ravit, B., Elgersma, K., 2005. Feedback in the plant-soil system. Annu. Rev. Env. Resour. 30, 75–115.
Falkner, G., Obrdlik, P., Castella, E., Speight, M.C.D., 2001. Shelled Gastropoda of Western Europe. Friedrich-Held-Gesellschaft, München.
Frömming, E., 1954. Biologie der mitteleuropäischen Landgastropden. Duncker und Humblot, Berlin.
Gerber, E., Krebs, C., Murrell, C., Moretti, M., Rocklin, R., Schaffner, U., 2008. Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. Biol. Conserv. 141, 646–654.
Grimshaw, H.M., 1989. Analysis of soils. In: Allen, S.E. (Ed.), Chemical Analysis of Ecological Materials. Blackwell, Oxford, UK, pp. 7–45.
Hawkins, J.W., Lankester, M.W., Nelson, R.R.A., 1998. Sampling terrestrial gastropods using cardboard sheets. Malacologia 39, 1–9.
Hejda, M., Pysek, P., 2006. What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? Biol. Conserv. 132, 143–152.
Hejda, M., Pysek, P., Voitech, J., 2009. Impact of invasive plants on the species richness, diversity and composition of invaded communities. J. Ecol. 97, 393–403.
Horsak, M., Cernohorsky, N., 2008. Mollusc diversity patterns in Central European fens: hotspots and conservation priorities. J. Biogeogr. 35, 1215–1225.
Horsak, M., Chytry, M., Danihelka, J., Koci, M., Kubesova, S., Lososova, Z., Otypkova, Z., Tichy, L., 2010. Snail faunas in the Southern Ural forests and their relations to vegetation: an analogue of the Early Holocene assemblages of Central Europe? J. Mollus. Stud. 76, 1–10.
Horsak, M., Hajek, M., 2003. Composition and species richness of molluscan communities in relation to vegetation and water chemistry in the western Carpathian spring fens: the poor-rich gradient. J. Mollus. Stud. 69, 349–357.
Hulme, P.E., Bremner, E.T., 2006. Assessing the impact of *Impatiens glandulifera* on riparian habitats: Partitioning diversity components following species removal. J. Appl. Ecol. 43, 43–50.
Johannessen, L.E., Solhøy, T., 2001. Effects of experimentally increased calcium levels in the litter on terrestrial snail populations. Pedobiologia 45, 234–242.
Kappes, H., Lay, R., Topp, W., 2007. Changes in different trophic levels of litter-dwelling macrofauna associated with giant knotweed invasion. Ecosystems 10, 734–744.
Kappes, H., Jordaens, K., Hendrickx, F., Maelfait, J.P., Lens, L., Backeljau, T., 2009. Response of snails and slugs to fragmentation of lowland forests in NW Germany. Landscape Ecol. 24, 685–697.
Kerney, M.P., Cameron, R.A.D., Jungbluth, J.H., 1983. Die Landschnecken Nord- und Mitteleuropas. Parey, Hamburg und Berlin.
Küng, J. 2010. Verändert der invasive Neophyt *Impatiens glandulifera* die natürliche Boden-Samenbank in Wäldern der Nordwestschweiz? MSc-thesis, University of Basel.
Labaune, C., Magnin, F., 2001. Land snail communities in Mediterranean upland grasslands: the relative importance of four sets of environmental and spatial variables. J. Mollus. Stud. 67, 463–474.
Lookwood, J.L., Hoopes, M.P., Marchetti, M.P., 2007. Invasion Ecology. Blackwell, Oxford.
Lopezaraiza-Mikel, M.E., Hayes, R.B., Whalley, M.R., Memmott, J., 2007. The impact of an alien plant on a native plant-pollinator network: an experimental approach. Ecol. Lett. 10, 539–550.
Martin, K., Sommer, M., 2004. Relationships between land snail assemblage patterns and soil properties in temperate-humid forest ecosystems. J. Biogeogr. 31, 531–545.
Mason, C.R., 1970. Snail populations, beech litter production and the role of snails in litter decomposition. Oecologia 5, 215–239.
Maule, H., Andrews, M., Watson, C., Cherrill, A.J., 2000. Distribution, biomass and effect on native species of *Impatiens glandulifera* in a deciduous woodland in northeast England. Asp. Appl. Biol. 58, 31–38.
Meteo Swiss, 2009. Normwerte 1961–90. Federal Office of Meteorology and Climatology MeteoSwiss (accessed 10.11.10.). http://www.meteoschweiz.ch.
Mueller-Dombois, D., Ellenberg, H., 2002. Aims and Methods of Vegetation Ecology. Blackburn Press, New Jersey.
Munoz Valles, S., Gallego Fernandez, J.B., Dellafiore, C., Cambrolle, J., 2011. Effects on soil, microclimate and vegetation of the native-invasive *Retama monosperma* (L.) in coastal dunes. Plant Ecol. 212, 169–179.
Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. In: Sparks, D.L. (Ed.), Methods of Soil Analysis. Part 3: Chemical Methods. Madison,, WI, USA, pp. 961–1010.
Nienhuis, C.M., Dietzsch, A.C., Stout, J.C., 2009. The impacts of an invasive alien plant and its removal on native bees. Apidologie 40, 450–463.
Nobis, M., 2008. Invasive Neophyten auch im Wald? Wald und Holz 8, 46–49.
Pejchar, L., Mooney, H.A., 2009. Invasive species, ecosystem services and human well-being. Trends Ecol. Evol. 24, 497–504.
Pysek, P., Vojtech, J., Hulme, P.E., Pergel, J., Hejda, M., Schaffner, U., Vila, M., 2012. A global assessment of invasive plant impacts on resident species, communities

and ecosystems: the interaction of impact measures, invading species' traits and environment. Glob. Change Biol. 18, 1725–1737.
R Development Core Team, 2010. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Reinhart, K.O., Callaway, R.M., 2006. Soil biota and invasive plants. New Phytol. 170, 455–457.
Saetersdal, M., Gjerde, I., Blom, H.H., Ihlen, P.G., Myrseth, E.W., Pommeresche, R., Skartveit, J., Solhøy, T., Aas, O., 2004. Vascular plants as a surrogate species group in complementary site selection for bryophytes, macrolichens, spiders, carabids, staphylinids, snails, and wood living polypore fungi in a northern forest. Biol. Conserv 115, 21–31.
Stoll, P., Gatzsch, K., Rusterholz, H.-P., Baur, B., 2012. Response of plant and gastropod species to knotweed invasion. Basic Appl. Ecol. 13, 232–240.
Stoll, P., Oggier, P., Baur, B., 2009. Population dynamics of six land snail species in experimentally fragmented grassland. J. Anim. Ecol. 78, 236–246.
ter Braak, C.J.E., Smilauer, P., 2002. CANOCO Reference Manual and User's Guide to CANOCO for Windows. In: Software for Canonical Community Ordination. Version 4.5. The Netherlands, and Microcomputer Power/Centre for Biometry, Wageningen/Ithaca, New York, USA.
Wäreborn, I., 1992. Changes in the land mollusc fauna and soil chemistry in an inland district in southern Sweden. Ecography 15, 62–69.
Wäreborn, I., 1979. Reproduction of two species of land snail in relation to calcium salts in the foerna layer. Malacologia 18, 177–180.
Walthert, L., Zimmermann, S., Blaser, P., Luster, J., Lüscher, P., 2004. Waldböden der Schweiz. In: Grundlagen und Region Jura, Band 1. Eidgenössische Forschungsanstalt WSL/Hep Verlag, Birmensdorf/Bern.
Wallwork, J.A., 1976. Ecology of Soil Animals. McGraw-Hill, New York.

CHAPTER 2

Invasion of an annual exotic plant into deciduous forests suppresses arbuscular mycorrhiza symbiosis and reduces performance of sycamore

Regina Ruckli, Hans-Peter Rusterholz and Bruno Baur

Forest Ecology and Management 318 (2014) 285–293

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Invasion of an annual exotic plant into deciduous forests suppresses arbuscular mycorrhiza symbiosis and reduces performance of sycamore maple saplings

Regina Ruckli, Hans-Peter Rusterholz *, Bruno Baur

Section of Conservation Biology, Department of Environmental Sciences, University of Basel, St. Johanns-Vorstadt 10, CH-4056 Basel, Switzerland

ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form 7 January 2014
Accepted 10 January 2014
Available online 14 February 2014

Keywords:
Acer pseudoplatanus
Disturbance
Forest ecosystem
Impatiens glandulifera
Plant–soil feedback
Survival

ABSTRACT

Invasive plants can disrupt associations between soil organisms and native trees which may result in altered ecosystem functions, both reduced biodiversity and timber production. We examined the effect of the invasive annual plant *Impatiens glandulifera* on the arbuscular mycorrhiza (AM) symbiosis and performance of *Acer pseudoplatanus* saplings at three different levels of disturbance in a controlled field experiment in a deciduous forest in Switzerland. A total of 1125 one-year-old *A. pseudoplatanus* saplings were planted either in plots invaded by *I. glandulifera*, in plots from which the invasive plant had been manually removed or in plots which were not yet colonised by the invasive plant. The 45 (3×15) plots were equally distributed over three forest areas which were differently affected by a wind throw 12 years prior to the experiment. Saplings including their full root systems were harvested after 3, 6 and 15 months. *I. glandulifera* reduced AM colonisation on *A. pseudoplatanus* saplings by 30–43%. Reduction in AM colonisation did not differ between harvesting time and was not affected by the level of forest disturbance. Saplings growing in invaded plots also showed a reduced root biomass and survival rate compared to saplings in the other plots. Increases in soil moisture, soil pH, available phosphorus and microbial activity found in plots invaded by *I. glandulifera* did not affect AM colonisation and performance of saplings. Our findings demonstrate that the spread of the invasive *I. glandulifera* in mixed deciduous forests negatively affects the symbiotic association between AM fungi and *A. pseudoplatanus* saplings and thus forest regeneration.

1. Introduction

The intentional and unintentional introduction of non-native species is considered as a major threat for native biodiversity (Pimentel et al., 2005; Pejchar and Mooney, 2009). Non-native plant species have the potential to affect an ecosystem by changing species diversity, community structure and interactions among organisms (Mooney and Hobbs, 2000; Kourtev et al., 2002; Stoll et al., 2012). Moreover, non-native plants may be able to change soil properties such as pH, moisture, and nutrient content (e.g. phosphorus and nitrogen) which in turn affect native biodiversity (Ehrenfeld et al., 2005; Munoz Valles et al., 2011).

Several factors are known to contribute to the invasion success of non-native plants in novel habitats. Environmental disturbance is a key factor facilitating the invasion and spread of non-native plant species into various habitats by changing ecosystem properties (Elton, 1958; D'Antonio et al., 1999; Hierro et al., 2006; Lookwood et al., 2007). Furthermore, there is growing evidence that invasive plants are able to alter the abundance and composition of soil microbial organisms and to disrupt symbiotic associations between soil fungi and host plants which increases the invasion success (Richardson et al., 2000; Reinhart and Callaway, 2006; Weidenhamer and Callaway, 2010).

Arbuscular mycorrhiza (AM) constitute the most abundant and oldest type of mycorrhiza symbiosis (Smith and Read, 2009). More than 80% of the vascular plants develop AM symbiosis with specific soil fungi that belong to the phylum *Glomeromycota* (Schussler et al., 2001). AM symbiosis is also a key interaction in tree species of different forest ecosystems (Wang and Qiu, 2006). AM symbiosis increases both soil nutrient and water uptake of host trees, strengthens pathogen resistance and protects the host plant during drought (Smith and Read, 2009). AM fungi facilitate the recruitment and establishment of tree seedlings (Brundrett, 1991; Kiers et al., 2000). Moreover, AM symbiosis plays a key role for the transport of resources within an ecosystem and is an important factor

* Corresponding author. Tel.: +41 61 267 08 50; fax: +41 61 267 08 32.
E-mail address: hans-peter.rusterholz@unibas.ch (H.-P. Rusterholz).

http://dx.doi.org/10.1016/j.foreco.2014.01.015
0378-1127/

determining the diversity of plant communities in space and time (Allen, 1991; Smith and Read, 2009; Johnson et al., 2012).

Several studies demonstrated that biennial and perennial invasive plants including *Alliaria petiolata* and *Tamarix* sp. reduce the colonisation rate of AM on native *Acer* and *Populus* trees (Roberts and Anderson, 2001; Stinson et al., 2006; Meinhardt and Gehring, 2012). Moreover, the invasive plant *A. petiolata* altered AM communities in forest soils (Barto et al., 2011). Thus, the disruption of AM symbiosis by invasive plants may have severe consequences for the performance of tree saplings.

In the last decade, the annual plant *Impatiens glandulifera* (Himalayan balsam) has increasingly invaded deciduous and coniferous forests disturbed by wind throws and/or intensive forest management in Central Europe (Nobis, 2008). *I. glandulifera* 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). It became naturalized and invasive in riparian habitats and disturbed areas (Hejda and Pysek, 2006). Relatively few studies investigated the potential impact of *I. glandulifera* on native biodiversity. The presence of *I. glandulifera* causes slight changes in the cover of plant species and shifts in the species composition in riparian habitats (Hejda and Pysek, 2006; Maule et al., 2000). *I. glandulifera* competes successfully with native plants for pollinators, which could lead to a reduced plant fitness (Chittka and Schurkens, 2001; Lopezaraiza-Mikel et al., 2007). In mixed deciduous forests, *I. glandulifera* affects the litter-dwelling gastropod community by increasing soil moisture and dampening daily soil temperature fluctuations (Ruckli et al., 2013).

In contrast to perennial invasive plants, relatively little is known concerning the impact of annual invasive plant species on the performance of tree saplings and the symbiosis between saplings and AM fungi. In the present study, the Sycamore maple (*Acer pseudoplatanus*), a common deciduous tree species in Central Europe, was used as a model species to study the impact of the invasive *I. glandulifera* on the symbiotic association between saplings and AM fungi. A balanced field experiment was designed with plots placed in patches of *I. glandulifera*, plots from which the invasive plant was regularly removed and plots which were not yet colonised by the invasive plant. The three treatment plots were equally distributed over three forest areas that were differently affected by a wind throw 12 years prior to the experiment, creating a natural gradient of disturbance. One-year-old *A. pseudoplatanus* saplings planted in the experimental plots were harvested after 3, 6 and 15 months to assess *I. glandulifera*-induced changes in AM colonisation, survival and biomass of saplings. The study also examined whether the presence of *I. glandulifera* alters chemical and biochemical soil properties which in turn may affect AM colonisation and the performance of *A. pseudoplatanus* saplings. The following hypotheses were tested: (1) The invasion of *I. glandulifera* reduces the AM colonisation of fine roots of *A. pseudoplatanus* saplings. (2) The invasive plant-induced reduction of AM colonisation increases with time. (3) Survival and biomass of *A. pseudoplatanus* saplings are reduced in the presence of the invasive plant. (4) Intensities of former forest disturbance by the wind throw amplifies the impact of *I. glandulifera* on AM colonisation of *A. pseudoplatanus* saplings.

2. Materials and methods

2.1. Field experiment

The experiment was carried out in three areas (each measuring 50 × 180 m) in a mixed deciduous forest 15 km south of Basel, northern Switzerland (47°26′N, 7°33′E). In this region, the annual temperature averages 9.6 °C and the annual precipitation is 1021 mm (Meteo Swiss, 2012). The three areas (situated within 1 km^2) were differently affected by the windstorm Lothar in 1999. Eight years after this storm, the disturbance intensity in the three areas was assessed by measuring the canopy closure of the remaining forest trees using a spherical crown densitometer (Forest suppliers Inc., US). One area with a canopy closure of 80% was considered as low disturbed, another area with a canopy cover of 50% as moderately disturbed, and the remaining area with a canopy closure of 2.5% as heavily disturbed (Appendix A). *I. glandulifera* started to invade the forest shortly after the storm in spring 2000.

In spring 2008 (8 years after invasion), five homogenous patches of *I. glandulifera* were selected in each area. The patches were situated 5–10 m apart from each other. In each patch, two 5 × 5 m plots with similar *I. glandulifera* cover adjacent to each other were installed. In one of the two plots all *I. glandulifera* individuals were removed by hand (hereafter referred to as removed) every spring in the years 2008–2012. The other plot was left invaded by *I. glandulifera* (hereafter referred to as invaded). As an additional control, six 5 × 5 m control plots were selected that were not yet invaded by *I. glandulifera* in close proximity (7–20 m) to the experimental plots in each area (referred to as uninvaded). This allowed to control for the slight mechanical disturbance of the soil by removing *I. glandulifera*. To prevent colonisation of *I. glandulifera* in the not yet invaded plots, all invasive plants growing in close proximity to these plots were removed five times per year from 2008 to 2012. Thus, the experimental setup consisted of 45 treatment plots (15 invaded, 15 removed, 15 uninvaded) equally distributed over the three areas, which were differently affected by the storm.

A total of 1125 one-year-old *A. pseudoplatanus* saplings (25 saplings in each plot) obtained from a commercial forest nursery (Emme-Forstbaumschulen AG; seed origin: Hägendorf, Switzerland) were planted on 16 April 2011. To minimise potential edge effects, the saplings were planted in areas of 1 m^2 which at least 1 m apart from the plot border. Fine roots of the *A. pseudoplatanus* saplings were pruned prior to planting to avoid transplanting AMF from the nursery. Saplings of the same height (24–26 cm) were planted in the different plots.

2.2. AM colonisation and performance of A. pseudoplatanus saplings

To assess the effect of *I. glandulifera* on AM colonisation, survival, and root and shoot biomass of *A. pseudoplatanus* saplings, two saplings in each plot were harvested after 3 months (July 2011), 6 months (October 2011) and 15 months (July 2012) yielding a total of 30 saplings for each treatment plot at each sampling date. The saplings were carefully dug out with the entire root system and put in plastic bags together with soil of the corresponding plots. The number of viable and dead *A. pseudoplatanus* saplings was recorded at each sampling date. At the same time, the number of *I. glandulifera* individuals were counted in one subplot measuring 0.25 m^2 in each plot invaded by the invasive plant and their biomass was assessed (n = 15 subplots, Appendix A).

In the laboratory, sapling roots were separated from shoots and the roots were washed free of soil and organic debris. Any adhering material was removed with forceps. The fine roots were preserved in a 50% ethanol solution until AM determination. To estimate the percentage of AM colonisation on fine roots, a weighed fine root subsample of each sapling (range: 0.09–0.12 g) was cleared in 10% KOH (20 min, 121 °C) and stained for 3.5 min in a 5% ink-vinegar solution (ink; Pelikan blue, apple vinegar; 5% acetic acid) following Vierheilig et al. (1998). After staining, the percentage of AM colonisation was measured using the gridline intersection method of McGonigle et al. (1990). At least 100 root intersections were

checked from each *A. pseudoplatanus* sapling. The dry weights of roots and shoots were determined after oven drying (48 h, 60 °C).

2.3. Chemical soil properties and soil enzyme activities

To evaluate any potential influence of soil properties on AM colonisation, survival and biomass of *A. pseudoplatanus* saplings, five randomly chosen soil samples were collected in each plot using a metal cylinder (soil depth: 5 cm; soil volume: 100 cm^3) on the same days the saplings were harvested (in July 2011, October 2011 and July 2012). The five soil samples of a plot were mixed and transported on ice to the laboratory where they were sieved (mesh width 2 mm). A subsample was stored at −80 °C for analysis of enzyme activities.

Soil moisture content (%) was determined using the fresh to dry weight ratio. Soil pH was assessed in distilled water (1:2.5 soil:-water; Allen, 1989). Total soil organic matter content (SOM) was determined as loss-on-ignition of oven-dried soil at 700 °C for 23 h (Allen, 1989). Total phosphorus content and plant available phosphorus content of the soil were assessed using standard methods (Sparks et al., 1996).

Invasive plants have the potential to affect soil enzymes. Phosphatases are essential enzymes for the mineralisation of phosphorus which is important for AMF-development and performance of the host plant (Allen, 1991). Freezing and thawing does not affect soil enzyme activity within similar types of forest soil (Kissling et al., 2009). Prior to the analyses, the frozen soil samples were thawed and stored at 4 °C. All analyses were conducted within 7 days after thawing. The activities of phosphomonoesterase (EC 3.1.3.2) were measured using the p-nitrophenyl-ester based assay (Tabatabai and Bremner, 1969). 1 g moist soil were mixed with 4 ml of modified universal buffer (MUB, pH 6.5) and incubated for 1 h with 1 ml pNP (p-nitrophenyl phosphate, 15 mM) at 37 °C. Then 1 ml of $CaCl_2$ (0.5 M) was added and the reaction was terminated by adding 8 ml NaOH (0.5 M). The soil suspensions were centrifuged for 15 min at 4000 rpm and the p-nitrophenol (pNP) in the supernatant was measured spectrophotometrically at 400 nm.

Fluorescein diacetate (FDA) hydrolysis is a surrogate for both total microbial activity and amount of active fungi (Schnürer and Rosswall, 1982). We therefore assessed the effect of *I. glandulifera* on FDA hydrolysis by using the slightly modified method of Dick et al. (1996). 1 g of sieved field-moist soil was mixed with 10 ml of sodium phosphate buffer (6.0 mM, pH 7.6). To start the reaction 100 µl FDA (4.8 mM) was added. After an incubation time of 2 h at 37 °C, 10 ml of acetone was added to stop the reaction. The soil suspensions were centrifuged for 15 min at 4000 rpm and the absorbance of the supernatant was measured spectrophotometrically at 490 nm. For all enzymes, three replicates of each sample and substrate-free controls were analysed.

2.4. Statistical analyses

All statistical analyses were calculated using R 2.15.1 (R Development Core Team, 2012). Pearson correlation analysis showed that total soil phosphorus and plant available soil phosphorus were intercorrelated ($r = 0.42$, $n = 135$, $P < 0.001$). Consequently, plant available soil phosphorus can be considered as surrogate for the total soil phosphorus content. To avoid spatial pseudoreplication, the following analyses were performed with the mean values of each plot. Linear mixed models for temporal pseudoreplication (LME) were used to analyse the effects of treatment, disturbance intensity, harvesting time and assessed soil properties on the AM colonisation. Harvesting time and treatment (invaded, removed, uninvaded) were included as fixed factors, plot nested in time as random factor, and disturbance intensity and soil properties as cofactors. To assess the effect of the invasive plant on survival and different biomass measures (total, shoot and root biomass) of *A. pseudoplatanus* saplings, a similar LME was used, but AM colonisation rate was added as an additional cofactor. Harvesting time and treatment (invaded, removed, uninvaded) were included as fixed factors and plot nested in time as random factor. To examine the effect of *I. glandulifera* on the root biomass of *A. pseudoplatanus* saplings at the three sapling dates, an ANCOVA was used with treatment as factor and intensity of disturbance and soil properties as cofactors.

Linear regression analyses were applied to evaluate the effects of *I. glandulifera* density on AM colonisation, root and shoot biomass and survival rate of the saplings. To assess the effect of the invasive plant on chemical soil properties, LME was used with treatment and harvesting time as fixed factors and plot nested in time as random factor and intensity of disturbance and selected soil properties as cofactors. For biochemical properties (soil enzyme activity), similar LME were used, but the chemical soil

Table 1
Summary of LME analyses on AM colonisation, survival, total biomass and shoot and root biomass of *A. pseudoplatanus* saplings (Significant *P*-values (<0.05) are printed in bold).

	AM colonisation			Survival			Total biomass			Shoot biomass			Root biomass		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Treatment (T)	2,106	328.52	**<.0001**	2,102	44.91	**<.0001**	2,102	3.55	**0.0323**	2,105	1.39	0.2536	2,103	4.40	**0.0146**
Intensity of disturbance (Id)	1,106	1.33	0.2517	1,102	140.61	**<.0001**	1,102	3.74	0.0559	1,105	4.86	**0.0296**	1,103	1.48	0.2271
Harvesting time (Ht)	2,8	0.17	0.8438	2,8	18.39	**0.0010**	2,8	8.41	**0.0108**	2,105	14.55	**0.0022**	2,8	5.06	**0.0381**
AM colonisation (%)	–[B]	–[B]	–[B]	1,102	2.76	0.0997	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]
Soil moisture (%)	–[A]	–[A]	–[A]	1,102	0.01	0.9428	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]
Soil pH	1,106	2.39	0.1249	1,102	0.27	0.5995	1,102	2.47	0.1187	1,105	3.22	0.0755	1,103	1.33	0.2507
Total soil organic matter (%)	–[A]	–[A]	–[A]	1,102	3.57	0.0617	1,102	4.21	**0.0426**	1,105	2.72	0.1019	1,103	3.87	0.0518
Plant available phosphorus[a]	1,106	2.22	0.1395	–[A]	–[A]	–[A]	1,102	1.14	0.2862	–[A]	–[A]	–[A]	1,103	2.36	0.1271
PME activity[b]	–[A]	–[A]	–[A]	1,102	1.910	0.1700	1,102	0.52	0.4704	1,105	2.15	0.1453	1,103	0.016	0.8988
Hydrolyse activity of FDA[c]	1,106	1.32	0.2528	–[A]	–[A]	–[A]	1,102	1.24	0.2682	1,105	1.23	0.2695	–[A]	–[A]	–[A]
Soil moisture × T	–[A]	–[A]	–[A]	2,102	1.475	0.2336	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]
Soil pH × T	–[A]	–[A]	–[A]	2,102	2.789	0.0662	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	2,103	1.93	0.1494
Total soil organic matter × T	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	2,102	1.39	0.2535	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]
PME activity × T	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	2,102	3.33	**0.0395**	2,105	1.37	0.2566	2,103	2.79	0.0660
Hydrolyse activity of FDA × T	2.106	1.86	0.1605	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]	–[A]
Id × T	2,106	3.53	**0.0329**	2,102	2.70	0.0722	2,102	3.18	**0.0455**	2,105	2.48	0.0889	2,103	2.63	0.0768
Ht × T	4,106	20.98	**<.0001**	4,102	0.371	0.8291	4,102	1.62	0.1730	4,105	0.72	0.5776	4,103	2.37	0.0569

[A] Cofactor was excluded from the model.
[B] Not included in the model.
[a] PLANT available soil phosphorus; µg PSO_4 g^{-1} soil.
[b] PME (phosphomonoesterase) activity; mmol pNP g^{-1} h^{-1}.
[c] Hydrolyse activity of FDA (fluorescein diacetate); µg fluorescein g^{-1} soil h^{-1}.

properties were added as additional cofactors. If necessary, data were log- or arsin-transformed to obtain normally distributed residuals. Statistical models were stepwise reduced by deleting non-significant interactions as recommended by Crawley (2007).

3. Results

3.1. AM colonisation of A. pseudoplatanus saplings

Invasion of *I. glandulifera* resulted in a significant reduction of AM colonisation on the fine roots of *A. pseudoplatanus* saplings (Table 1 and Fig. 1). In general, AM colonisation was 30–43% lower on saplings growing in plots with the invasive plant than on saplings growing in plots without *I. glandulifera*. Surprisingly, AM colonisation of *A. pseudoplatanus* saplings was not affected by the harvesting time. The significant interaction between treatment and harvesting time resulted from a lower AM colonisation on saplings in plots from which the invasive plant had been removed compared to saplings in uninvaded plots sampled after 3 and 6 months (Fig. 1). After 15 months, however, AM colonisation on saplings did not differ between plots from which *I. glandulifera* had been removed and uninvaded plots (Fig. 1). The density of *I. glandulifera* did not affect AM colonisation (linear regression: $R^2 = 0.030$, $F_{1,43} = 2.38$, $P = 0.120$).

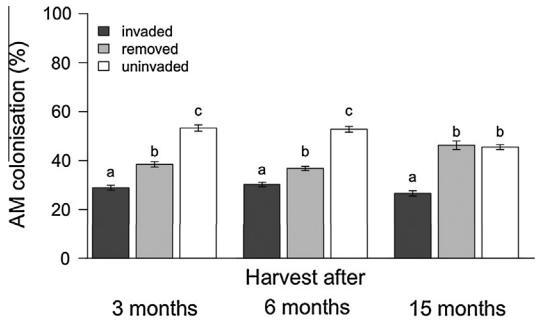

Fig. 1. AM colonisation (%) of *Acer pesudoplatanus* saplings growing in plots invaded by *Impatiens glandulifera*, in plots from which the invasive plant had been removed and in uninvaded plots. Saplings were harvested after 3, 6 and 15 months. Mean values ± SE are presented ($n = 15$ in each case). Different letters indicate significant differences among treatments (invaded, removed, uninvaded) within each harvesting time (Tukey's HSD, $p < 0.05$).

3.2. Survival and biomass of A. pseudoplatanus saplings

Invasion of *I. glandulifera* reduced the survival of *A. pseudoplatanus* saplings (Table 1, Fig. 2). After 15 months, the survival rate of saplings growing in invaded plots was 32% lower than those of saplings growing in plots in which *I. glandulifera* had been removed and in uninvaded plots (Fig. 2). The density of *I. glandulifera* did not affect the survival rate of saplings (linear regression: $R^2 = 0.012$, $F_{1,43} = 0.54$, $P = 0.467$).

The total biomass of *A. pseudoplatanus* saplings was reduced in plots invaded by *I. glandulifera* compared to those of saplings growing in plots from which the invasive plant had been removed and in uninvaded plots (Table 1, Appendix B). This was primarily a result of the reduced root biomass, because the invasive plant did not affect the saplings' shoot biomass (Table 1). Overall, the root biomass of *A. pseudoplatanus* saplings was 12–18% lower in plots with the invasive plant than the root biomass of saplings in plots in which the invasive plant had been removed and in uninvaded plots (Appendix B). Total biomass, root and shoot biomass of *A. pseudoplatanus* increased with duration of the experiment (expressed by harvesting time; Table 1, Appendix B). After 15 months, the root biomass of *A. pseudoplatanus* saplings was reduced by 20–31% in plots invaded by *I. glandulifera* compared to those in plots from which the plant had been removed and in uninvaded plots (ANCOVA; $F_{2,39} = 4.81$, $P = 0.013$). However, after 3 and 6 months, the root biomass did not differ among treatments (3 months: $F_{2,39} = 2.62$, $P = 0.096$; 6 months: $F_{2,39} = 1.80$, $P = 0.177$). The total biomass of saplings was also influenced by soil organic matter as indicated by the LME-analyses (Table 1). Phosphomonoesterase (PME) activity did not directly affect the total biomass. However, the significant interaction between PME activity and treatment indicates that total sapling biomass was differently affected by the PME activity in the different treatments (Table 1). The total biomass of saplings was not influenced by the density of the invasive plant (linear regression: $R^2 = 0.020$, $F_{1,43} = 0.11$, $P = 0.742$).

3.3. Soil properties and soil enzyme activity

The presence of *I. glandulifera* changed soil moisture, soil pH, plant available soil phosphorus and the hydrolyse of fluorescein diacetate (FDA) (Table 2). In contrast, total soil organic matter and activity of PME were not affected by the invasion of *I.*

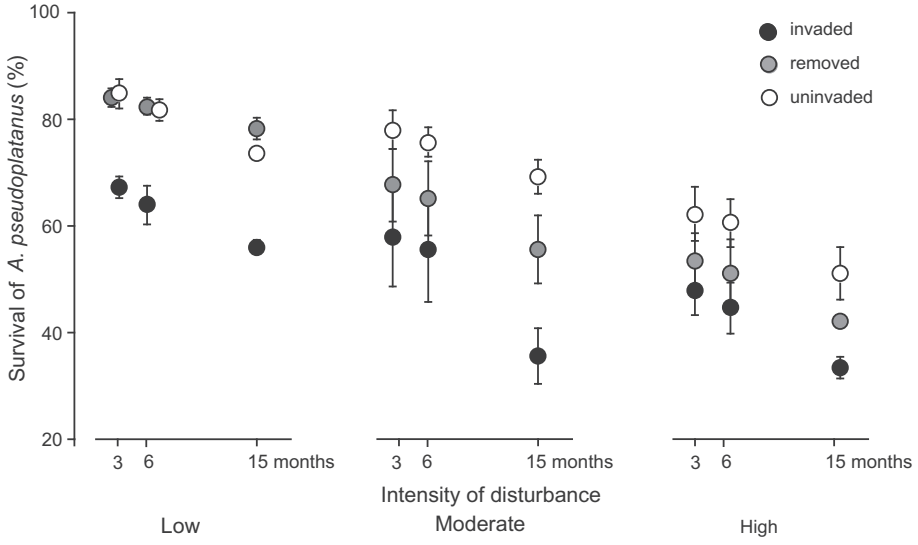

Fig. 2. Survival (%) of *Acer pseudoplatanus* saplings in plots invaded by *Impatiens glandulifera* (black circles), in plots from which the invasive plant had been removed (grey circles) and in uninvaded plots (open circles). The different plots were equally distributed in three differently disturbed forest areas. The percentage of surviving saplings was recorded after 3 months, 6 months and 15 months. (Mean values ± SE are presented ($n = 5$, in each case).

Table 2
Summary of LME analyses on different chemical and biochemical (enzyme) soil properties (Significant *P*-values (<0.05) are printed in bold).

	Soil moisture			Soil pH			Total soil organic matter			Plant av. soil phosphorus			PME activity			Hydrolyse activity of FDA		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Treatment (T)	2,107	15.03	**<.0001**	2,107	15.34	**<.0001**	2,105	1.77	0.1741	2,110	5.22	**0.0068**	2,108	0.63	0.5322	2,106	7.73	**0.0007**
Intensity of disturbance (Id)	1,107	29.74	**<.0001**	1,107	0.27	0.6040	1,105	6.46	**0.0124**	1,101	11.73	**<.0001**	1,108	0.71	0.4012	1,106	4.74	**0.0317**
Sampling date (S)	2,8	12.55	**0.0034**	2,8	8.15	**0.0118**	2,8	2.47	0.1464	2,8	130.57	**<.0001**	2,8	17.21	**0.0013**	2,8	5.31	**0.0341**
Soil moisture (%)	_B	_B	_B	_B	_B	_B	1,105	31.46	**<.0001**	_A	_A	_A	1,108	4.12	**0.0446**	1,106	5.13	**0.0256**
Soil pH	_B	_B	_B	_B	_B	_B	1,105	43.94	**<.0001**	2,110	2.76	0.0990	1,108	8.01	**0.0055**	1,106	4.12	**0.0448**
Total soil organic matter (%)	_B	_B	_B	1,107	39.62[B]	**<.0001**	_B	_B	_B	_B	_B	_B	1,108	16.34	**0.0001**	_A	_A	_A
Plant av. soil phosphorus [a]	_B	_B	_B	_A	_A	_A	_A	_A	_A	_B	_B	_B	_A	_A	_A	1,106	0.78	0.3772
PME activity [b]	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_A	_A	_A
Hydrolyse activity of FDA [c]	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_B	_A	_A	_A	_B	_B	_B
Soil moisture × T	_B	_B	_B	_A	_A	_A	2,105	1.37	0.2571	_A	_A	_A	_A	_A	_A	_A	_A	_A
Soil pH × T	_B	_B	_B	_B	_B	_B	2,105	5.71	0.0044	_A	_A	_A	1,108	1.39	0.2528	_A	_A	_A
Total soil organic matter × T	_B	_B	_B	2,107	1.69	0.1887	_B	_B	_B	_A	_A	_A	_A	_A	_A	_A	_A	_A
Plant av. phosphorus × T	_B	_B	_B	_A	_A	_A	_A	_A	_A	_B	_B	_B	_A	_A	_A	2,106	4.23	**0.0171**
Id × T	2,107	0.635	0.5320	2,107	1.85	01617	2,105	0.09	0.9100	2,110	2.95	0.0560	2,108	0.20	0.8111	2,106	0.53	0.5912
S × T	4,107	0.794	0.5314	4,107	2.05	0.0924	4,105	0.95	0.4382	4,110	0.36	0.8349	4,108	0.95	0.4352	4,106	1.08	0.3688

[A] cofactor was excluded from the model.
[B] not included in the model.
[a] Plant available soil phosphorus; μg $PSO_4\,g^{-1}$ soil.
[b] PME (phosphomonoesterase) activity; mmol pNP $g^{-1}\,h^{-1}$.
[c] Hydrolyse activity of FDA (fluorescein diacetate); mg fluorescein g^{-1} soil h^{-1}.

glandulifera (Table 2). In general, soil moisture was 15% higher in invaded than in uninvaded plots (Table 3). Soil pH was less acid in plots with *I. glandulifera* (mean ± se: 4.70 ± 0.03) than in plots from which the invasive plant had been removed (4.58 ± 0.02) and in uninvaded plots (4.52 ± 0.03). The difference in soil pH between invaded and uninvaded plots was more pronounced in July and October 2011 than in July 2012 (Table 3). Furthermore, soil pH was negatively affected by total soil organic matter (Table 2). Plant available soil phosphorus was 13% and 20% higher in plots with *I. glandulifera* than in plots in which the invasive plant had been removed and in uninvaded plots (Table 3). The amount of plant available soil phosphorus increased with time (Table 3). Hydrolyse activity of FDA, a surrogate for fungal biomass and total microbial activity (Adam and Duncan, 2001; Gaspar et al., 2001), was increased in the presence of the invasive plant (Table 2). Interestingly, the effect of *I. glandulifera* on FDA activity was more pronounced in autumn than in summer (Table 3). Furthermore, FDA activity was positively affected by soil moisture and soil pH (Table 2). There were no interactions between treatment and sampling date in any of the soil properties examined.

3.4. Effect of former forest disturbance

Intensity of former forest disturbance did not influence the AM colonisation of *A. pseudoplatanus* saplings. However, the significant interaction between disturbance intensity and treatment indicates that AM colonisation was differently affected by different disturbance intensity in the three treatments (Table 1). In contrast, sapling survival decreased with increasing intensity of forest disturbance (Table 1, Fig. 2). Furthermore, both total and shoot biomass of saplings increased with the intensity of former forest disturbance (Table 1, Appendix B), while root biomass was unaffected by the disturbance intensity (Table 1, Appendix B).

Soil moisture content and total soil organic matter increased with intensity of disturbance (Table 3). In contrast, plant available soil phosphorus content and FDA hydrolyse activity were negatively affected by disturbance intensity (Table 3). Soil pH and PME activity were not affected by the intensity of former forest disturbance.

4. Discussion

The present study demonstrates that the invasive annual plant *I. glandulifera* reduces AM colonisation of *A. pseudoplatanus* saplings. Furthermore, in the presence of *I. glandulifera*, saplings showed both reduced survival and root biomass compared to control saplings. The presence of the invasive plant also increased soil moisture, soil pH, plant available phosphorus and the hydrolysis activity of FDA.

Non-native plants increasingly invade deciduous forests (Essl et al., 2011; Godoy et al., 2011; Rusterholz et al., 2012) and there is growing evidence that non-native plants disturb AM associations of native tree species (Barto et al., 2011; Meinhardt and Gehring, 2012; Stinson et al., 2006). Most of the trees depend on AM symbiosis during the juvenile stage. The reduction of AM colonisation of *A. pseudoplatanus* saplings (30–43%) recorded in our study is similar to the reduction found in other studies that considered biennial and perennial invasive plants: 16–85% in saplings of various *Acer* sp. affected by the non-native *Alliaria petiolata* (Stinson et al., 2006; Barto et al., 2011), 25% in saplings of *Populus fremontii* influenced by invasive *Tamarix* sp. (Meinhardt and Gehring, 2012) and 50–60% in juvenile *Plantago lanceolata*, *Lotus corniculatus* and *Trifolium pratense* plants affected by *I. glandulifera* (Tanner and Gange, 2013).

Table 3
Chemical and biochemical soil properties in the three treatment plots (invaded by *Impatiens glandulifera*, invasive plant removed, and uninvaded) equally distributed in low, moderately and highly disturbed forest areas. Soil samples were taken in July and October 2011 and in July 2012.

Sampling date	Intensity of former forest disturbance								
	Low			Moderate			High		
	Invaded	Removed	Uninvaded	Invaded	Removed	Uninvaded	Invaded	Removed	Uninvaded
July 2011									
Soil moisture (%)	26.38 ± 0.84	26.85 ± 1.43	26.51 ± 1.10	27.62 ± 1.96	28.20 ± 2.04	22.49 ± 1.31	32.06 ± 1.01	27.77 ± 0.82	26.59 ± 3.64
Soil pH	4.80 ± 0.09	4.56 ± 0.10	4.71 ± 0.02	4.74 ± 0.04	4.72 ± 0.15	4.51 ± 0.06	4.72 ± 0.06	4.72 ± 0.05	4.57 ± 0.07
Total soil organic matter (%)	9.51 ± 0.48	12.66 ± 1.53	10.49 ± 0.45	13.44 ± 1.92	13.28 ± 2.37	10.69 ± 0.81	14.00 ± 1.43	10.85 ± 0.84	13.00 ± 3.04
Plant av. soil phosphorus[a]	46.23 ± 6.41	50.99 ± 5.30	42.29 ± 10.99	35.72 ± 5.82	34.92 ± 7.54	31.59 ± 8.26	32.72 ± 13.69	19.91 ± 5.34	21.55 ± 3.86
PME activity[b]	7.34 ± 0.43	8.23 ± 0.55	5.77 ± 0.79	4.92 ± 0.99	3.53 ± 0.60	3.40 ± 0.48	5.28 ± 0.27	5.25 ± 1.03	4.73 ± 0.93
Hydrolyse activity of FDA[c]	107.74 ± 8.16	122.84 ± 7.52	98.10 ± 12.52	119.20 ± 5.05	92.71 ± 16.23	107.27 ± 9.99	131.14 ± 3.61	104.93 ± 7.25	100.21 ± 8.90
October 2011									
Soil moisture (%)	24.17 ± 1.02	23.49 ± 0.95	20.63 ± 0.53	28.06 ± 2.03	24.04 ± 1.23	19.41 ± 0.79	31.97 ± 0.94	32.34 ± 1.78	25.78 ± 1.70
Soil pH	4.84 ± 0.21	4.46 ± 0.08	4.60 ± 0.02	4.64 ± 0.05	4.48 ± 0.11	4.38 ± 0.12	4.83 ± 0.07	4.65 ± 0.02	4.54 ± 0.12
Total soil organic matter (%)	12.59 ± 0.83	12.61 ± 1.06	10.25 ± 0.42	14.91 ± 1.18	12.95 ± 1.87	13.09 ± 1.47	12.13 ± 1.34	12.41 ± 1.89	11.14 ± 0.50
Plant av. soil phosphorus[a]	55.51 ± 10.41	43.93 ± 9.15	28.67 ± 7.30	47.33 ± 6.05	30.04 ± 5.84	27.82 ± 6.01	42.21 ± 3.23	27.92 ± 4.76	30.34 ± 3.65
PME activity[b]	10.42 ± 0.79	9.61 ± 0.23	8.27 ± 0.70	7.20 ± 0.61	7.06 ± 1.53	8.48 ± 0.94	10.30 ± 1.23	8.97 ± 0.71	8.71 ± 0.96
Hydrolyse activity of FDA[c]	123.35 ± 9.95	98.94 ± 6.53	89.31 ± 10.51	121.46 ± 8.49	100.18 ± 15.40	111.93 ± 4.72	108.20 ± 7.65	95.78 ± 5.80	86.86 ± 11.15
July 2012									
Soil moisture (%)	30.32 ± 1.24	29.41 ± 0.78	28.53 ± 1.35	30.94 ± 1.63	27.76 ± 1.15	22.76 ± 3.56	34.17 ± 1.07	31.13 ± 1.42	32.26 ± 2.23
Soil pH	4.65 ± 0.11	4.63 ± 0.08	4.61 ± 0.03	4.53 ± 0.06	4.50 ± 0.07	4.38 ± 0.10	4.59 ± 0.04	4.56 ± 0.03	4.40 ± 0.05
Total soil organic matter (%)	10.50 ± 0.71	9.91 ± 0.59	9.77 ± 0.32	10.61 ± 1.08	9.39 ± 1.53	12.31 ± 2.01	13.29 ± 1.74	12.16 ± 1.13	11.76 ± 1.43
Plant av. soil phosphorus[a]	150.32 ± 18.40	130.24 ± 14.51	103.97 ± 14.69	115.48 ± 21.94	119.56 ± 11.66	111.22 ± 5.56	115.41 ± 10.31	104.47 ± 18.20	115.96 ± 9.63
PME activity[b]	5.51 ± 1.89	6.16 ± 2.21	5.12 ± 0.67	5.91 ± 0.86	7.75 ± 2.12	7.35 ± 2.27	8.04 ± 1.51	8.56 ± 1.53	8.70 ± 1.06
Hydrolyse activity of FDA[c]	132.63 ± 11.03	144.99 ± 13.37	142.05 ± 7.37	127.97 ± 11.66	124.52 ± 8.26	70.35 ± 4.20	117.07 ± 6.21	102.05 ± 7.75	113.39 ± 9.20

Note: Means ± SE are given, $n = 5$ in each case.
[a] Plant available soil phosphorus; μg PSO_4 g^{-1} soil.
[b] PME (phosphomonoesterase) activity; mmol pNP g^{-1} h^{-1}.
[c] Hydrolyse activity of FDA (fluorescein diacetate); mg fluorescein g^{-1} soil h^{-1}.

The present study is the first that investigated the potential influence of an annual invasive plant on AM symbiosis. No seasonal variation in the extent of AM colonisation in *A. pseudoplatanus* saplings was recorded in the present study rejecting the second hypothesis. This unexpected result contradicts earlier findings that AM colonisation of tree saplings increases from early spring to summer and then declines in autumn (Brundrett and Kendrick, 1988; Cooke et al., 1992), or varies in the course of the year independent of external disturbances (Demars and Boerner, 1995; Escudero and Mendoza, 2005; Kabir et al., 1997). However, differences in experimental designs, sampling schedules and life form of the species examined should be taken in account when different studies are compared. It is possible that saplings were harvested before and after the seasonal peak of AM colonisation and therefore its seasonal variation could not be shown. The experimental approach used in the present study rules out potential differences in plant performance and in historical and initial conditions between invaded and uninvaded plots. In contrast, the majority of the other studies were field surveys in which focal trees were sampled both in invaded sites and uninvaded control sites in the neighbourhood. Controlled field experiments reveal more distinct results than field surveys disturbed by confounding factors (Hulme and Bremner, 2006; Lopezaraiza-Mikel et al., 2007; Nienhuis et al., 2009).

Allelopathic compounds released in the soil could be the mechanism by which *I. glandulifera* reduces AM colonisation on *A. pseudoplatanus* saplings. Indeed, shoots and roots of *I. glandulifera* contain napthoquinones (Lobstein et al., 2001), which show antimicrobial and antifungal effects (Ruckli et al., in press). Using the same plots of the field experiment as in the present study, napthoquinones were found in moderate quantities in the soil of plots invaded by *I. glandulifera* and in very low quantities in plots from which the invasive plant had been removed but not in soil of uninvaded plots (Ruckli et al., in press). The concentrations of these napthoquinones decreased after the senescence of *I. glandulifera* bioassays showed that they reduce the growth of mycorrhizal fungi mycelium (Ruckli et al., in press). These findings support the hypothesis that allopathic effects of *I. glandulifera* inhibit AM symbiosis. There is evidence from several studies on other invasive plants that allelopatic compounds disturb mycorrhizal symbiosis (Roberts and Anderson, 2001; Mummey and Rillig, 2006; Wolfe et al., 2008; Grove et al., 2012).

Light availability is an important factor for the development of AM symbiosis on tree species (e.g. McGee and Furby, 1992; Gehring, 2004). In invaded forest areas, dense stands of *I. glandulifera* could reduce light availability for *A. pseudoplatanus* saplings. In the present study, the density of *I. glandulifera* was considered as a surrogate for light availability. However, regression analysis revealed that the density of *I. glandulifera* had no influence on the extent of AM colonisation. Thus, the reduced AM colonisation in invaded plots cannot be explained by differences in light conditions.

Invasive plants are often highly competitive and exhibit different strategies to affect the performance of native plants (Levine et al., 2003). For example, the invasive shrub *Lonicera maackii* decreased both the survival and growth rates of *Acer sacchacrum* and *Fagus americana* seedlings in a deciduous forest (Gorchov and Trisel, 2003), and the invasive grass *Lolium arundinaceum* and *Elaeagnus umbellata* reduced the root biomass of *Platanus occidentalis* (Orr et al., 2005). Similarly, in the present study, *I. glandulifera* reduced the survival rate and root biomass of *A. pseudoplatanus* saplings.

Out shading by invasive plants is assumed to be a key factor for the reduction of the performance of native trees (Gorchov and Trisel, 2003; Flory and Clay, 2010). Our study showed that the presence of *I. glandulifera* influenced both the survival and biomass of *A. pseudoplatanus*. However, the negative effect was not amplified by the density of the invasive plant. In contrast, Ammer et al. (2011) showed that the presence of *I. glandulifera* affected neither growth nor survival of *Abies alba* and *Picea abies* saplings. This discrepancy could be explained by species-specific responses or by the fact that control plots were already sparsely invaded by *I. glandulifera* in that study. In the present study, *I. glandulifera* influenced *A. pseudoplatanus* growth and survival even at low densities (38 individuals per m^2).

Invasive plant species have the potential to alter chemical and biochemical soil properties that are important for microbial and plant communities (Weidenhamer and Callaway, 2010). Increases in soil pH, soil moisture and plant available phosphorus were recorded in plots invaded by *I. glandulifera*. Other studies also showed that invasive plants increased soil moisture and soil pH (Suding et al., 2004; Li et al., 2006; Rodgers et al., 2008) as well as soil phosphorus content (Chapuis-Lardy et al., 2006; Thorpe et al., 2006; Niu et al., 2007). In the present study, the presence of *I. glandulifera* increased hydrolyse activity of FDA, which is a surrogate for both total microbial activity and saprophytic/arbuscular fungi biomass (Adam and Duncan, 2001; Gaspar et al., 2001). This finding is in line with the results of Sanon et al. (2009) showing an increased microbial activity (FDA) in soils invaded by *Acacia viridis*. However, the observed changes in soil properties caused by *I. glandulifera* did not affect AM colonisation and the performance of saplings. Generally, soil phosphorus is considered as key factor for the development of AM colonisation on host plants (e.g. Demiranda and Harris, 1994; Schroeder and Janos, 2005). It is possible that the difference in soil phosphorus content recorded between treatments in the present study was too small to influence AM colonisation. Alternatively, the effect of phosphorus on AM colonisation may be affected by species-specific characteristics of the host plant and environmental factors (Weber and Claus, 2000).

Forest disturbance is an additional factor that may influence AM colonisation of host trees. However, its effect may depend on the type and severity of disturbance, on the spatial scale and the kind of response of the AM host plant association (Jasper et al.,1991; Merryweather and Fitter, 1998; Titus and Leps, 2000). In the present study, however, former forest disturbance did not affect AM colonisation of the *A. pseudoplatanus* saplings. This could be explained by the fact that AM mycelium has the potential to regenerate within several months after a short-term disturbance (Jasper et al., 1991; Merryweather and Fitter, 1998).

The findings of the present study demonstrate that *I. glandulifera* negatively affects the performance of *A. pseudoplatanus* saplings in mixed deciduous forest. The combined effects of altered soil properties and decreased AM colonisation on saplings may influence forest regeneration after disturbance and reduce timber production. To avoid or reduce these negative effects on forest trees, it is recommended to remove *I. glandulifera* when the invasive plant is still in the colonising phase.

Acknowledgements

We thank Anette Baur, Evelyn Meyer and an anonymous reviewer for comments on the manuscript, Katharina Hesse, Esther Ruckli, Armando Lenz, Anette Baur, Jonas Küng, Samuel Lüdin and Martin Baur for field assistance. Financial support was received from the Velux Foundation, Zürich.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2014.01.015.

References

Adam, G., Duncan, H., 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol. Biochem. 33, 943–951.

Allen, M.F., 1991. The Ecology of Mycorrhizae. Cambridge University Press, Cambridge.

Allen, S.E., 1989. Chemical Analysis of Ecological Materials, second ed. Blackwell Scientific Publications, Oxford.

Ammer, C., Schall, P., Woerdehoff, R., Lamatsch, K., Bachmann, M., 2011. Does tree seedling growth and survival require weeding of Himalayan balsam *Impatiens glandulifera*? Eur. J. For. Res. 130, 107–116.

Barto, E.K., Antunes, P.M., Stinson, K., Koch, A.M., Klironomos, J.N., Cipollini, D., 2011. Differences in arbuscular mycorrhizal fungal communities associated with sugar maple seedlings in and outside of invaded garlic mustard forest patches. Biol. Invasions 13, 2755–2762.

Beerling, D.J., Perrins, J.M., 1993. *Impatiens glandulifera* Royle (*Impatiens roylei* Walp.). J. Ecol. 81, 367–382.

Brundrett, M.C., 1991. Mycorrhizas in natural ecosystems. Adv. Ecol. Res. 21, 171–313.

Brundrett, M.C., Kendrick, B., 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Can. J. Bot. 66, 1153–1173.

Chapuis-Lardy, L., Vanderhoeven, S., Dassonville, N., Koutika, L., Meerts, P., 2006. Effect of the exotic invasive plant *Solidago gigantea* on soil phosphorus status. Biol. Fertil. Soils 42, 481–489.

Chittka, L., Schurkens, S., 2001. Successful invasion of a floral market – an exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature 411, 653.

Cooke, M.A., Widden, P., Ohalloran, I., 1992. Morphology, incidence and fertilization effects on the vesicular–arbuscular mycorrhizae of *Acer saccarum* in a Quebec hardwood forest. Mycologia 84, 422–430.

Crawley, M.J., 2007. The R book. Wiley, Chichester.

D'Antonio, C.M., Dudley, T.L., Mack, M., 1999. Disturbance and biological invasions: direct effects and feedbacks. In: Walker, L.R. (Ed.), Ecosystems of Disturbed Ground. Elsevier, Amsterdam, pp. 413–452.

Demars, B.G., Boerner, R.E.J., 1995. Mycorrhizal dynamics of three woodland herbs of contradicting phenology along topographic gradients. Am. J. Bot. 82, 1426–1431.

Demiranda, J.C.C., Harris, P.J., 1994. Effects of soil-phosphorus on spore germination and hyphal growth of arbuscular mycorrhizal fungi. New Phytol. 128, 103–108.

Dick, R.P., Breakwell, D.P., Turco, R.F., 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran, J.W., Jones, A.J. (Eds.), Methods of assessing soil quality. SSSA special publication 49, Soil Science Society of America, Madison, pp. 247–271.

Ehrenfeld, J.G., Ravit, B., Elgersma, K., 2005. Feedback in the plant–soil system. Ann. Rev. Environ. Res. 30, 75–115.

Elton, C.S., 1958. The Ecology of Invasions by Animals and Plants. Methuen, London.

Escudero, V., Mendoza, R., 2005. Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. Mycorrhiza 15, 291–299.

Essl, F., Milasowszky, N., Dirnböck, T., 2011. Plant invasions in temperate forests: resistance or ephemeral phenomenon. Basic Appl. Ecol. 12, 1–9.

Flory, S.L., Clay, K., 2010. Non-native grass invasion suppresses forest succession. Oecologia 164, 1029–1038.

Gaspar, M.L., Cabello, M.N., Pollero, R., Aon, M.A., 2001. Fluorescein diacetate hydrolysis as a measure of fungal biomass in soil. Curr. Microbiol. 42, 339–344.

Gehring, C.A., 2004. Seed reserves and light intensity affect the growth and mycorrhiza development of the seedlings of an Australian rain-forest tree. J. Trop. Ecol. 20, 345–349.

Godoy, O., Saldano, A., Fuentes, N., Valladares, F., Gianoli, E., 2011. Forests are not immune to plant invasion: phenotypic plasticity and local adaptation allow *Prunella vulgaris* to colonize temperate evergreen rainforest. Biol. Invasions 13, 1611–1625.

Gorchov, D.L., Trisel, D.E., 2003. Competitive effects of the invasive shrub, *Lonicera maackii* (Rupr.) Herder (*Caprifoliaceae*), on the growth and survival of native tree seedlings. Plant Ecol. 166, 13–24.

Grove, S., Haubensak, K.A., Parker, I.M., 2012. Direct and indirect effects of allelopathy in the soil legacy of an exotic plant invasion. Plant Ecol. 213, 1869–1882.

Hejda, M., Pysek, P., 2006. What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? Biol. Conserv. 132, 143–152.

Hierro, H.L., Villarreal, D., Eren, Ö., Graham, J.M., Callaway, R.M., 2006. Disturbance facilitates invasions: the effects are stronger abroad than at home. Am. Nat. 168, 144–156.

Hulme, P.E., Bremner, E.T., 2006. Assessing the impact of *Impatiens glandulifera* on riparian habitats: partitioning diversity components following species removal. J. Appl. Ecol. 43, 43–50.

Jasper, D.A., Abbott, L.K., Robson, A.D., 1991. The effect of soil vesicular arbuscular mycorrhizal fungi in soil from different vegetation types. New Phytol. 118, 471–476.

Johnson, D., Martin, F., Cairney, J.W.G., Anderson, I.C., 2012. The importance of individuals: intraspecific diversity of mycorrhizal plants and fungi in ecosystems. New Phytol. 194, 614–628.

Kabir, Z., Ohalloran, I.P., Fyles, J.W., Hamel, C., 1997. Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: hyphal density and mycorrhizal root colonization. Plant Soil 192, 285–293.

Kiers, E.T., Lovelock, C.E., Krueger, E.L., Herre, E.A., 2000. Differential effects of tropical arbuscular fungal inocula on root colonisation and tree seedling growth: implications for tropical diversity. Ecol. Lett. 3, 106–113.

Kissling, M., Hegetschweiler, K.T., Rusterholz, H.-P., Baur, B., 2009. Short-term and long-term effects of human trampling on above-ground vegetation, soil density, soil organic matter and soil microbial processes in suburban beech forests. Appl. Soil Ecol. 42, 304–314.

Kourtev, P.S., Ehrenfeld, J.G., Haggblom, M., 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83, 3152–3166.

Levine, J.M., Vila, M., D'Antonio, C.M., Dukes, J.S., Grigulis, K., Lavorel, S., 2003. Mechanisms underlying the impacts of exotic plant invasions. Proc. R. Soc. Lond. Ser. B Biol. Sci. 270, 775–781.

Li, W.H., Zhang, C.B., Jiang, H.B., Xin, G.R., Yang, Z.Y., 2006. Changes in soil microbial community associated with invasion of the exotic weed, *Mikania micrantha* HBK. Plant Soil 281, 309–324.

Lobstein, A., Brenne, X., Feist, E., Metz, N., Weniger, B., Anton, R., 2001. Quantitative determination of naphthoquinones of *Impatiens* species. Phytochem. Anal. 12, 202–205.

Lookwood, J.L., Hoopes, M.P., Marchetti, M.O., 2007. Invasion Ecology. Blackwell, Oxford.

Lopezaraiza-Mikel, M.E., Hayes, R.B., Whalley, M.R., Memmott, J., 2007. The impact of an alien plant on a native plant-pollinator network: an experimental approach. Ecol. Lett. 10, 539–550.

Meteo Swiss, 2012. Normwerte 1961–1990. Federal Office of Meteorology and Climatology MeteoSwiss. <http://www.meteoschweiz.ch> (accessed 10.11.12).

Maule, H., Andrews, M., Watson, C., Cherrill, A.J., 2000. Distribution, biomass and effect on native species of *Impatiens glandulifera* in a deciduous woodland in northeast England. Aspects Appl. Biol. 58, 31–38.

McGee, P.A., Furby, J.H., 1992. Formation and structure of mycorrhizas of seedlings of coachwood (*Ceratopetalum apetalum*). Aust. J. Bot. 40, 291–304.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol. 115, 495–501.

Meinhardt, K.A., Gehring, C.A., 2012. Disrupting mycorrhizal mutualisms: a potential mechanism by which exotic tamarisk outcompetes native cottonwoods. Ecol. Appl. 22, 532–549.

Merryweather, J.W., Fitter, A.H., 1998. Patterns of arbuscular mycorrhiza colonisation of the roots of *Hyacinthoides non-scripta* after disruption of soil mycelium. Mycorrhiza 8, 87–91.

Mooney, H.A., Hobbs, R.J., 2000. Invasive Species in a Changing World. Island, Washington, DC.

Mummey, D.L., Rillig, M.C., 2006. The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant Soil 288, 81–90.

Munoz Valles, S., Gallego Fernandez, J.B., Dellafiore, C., Cambrolle, J., 2011. Effects on soil, microclimate and vegetation of the native-invasive *Retama monosperma* (L.) in coastal dunes. Plant Ecol. 212, 169–179.

Nienhuis, C.M., Dietzsch, A.C., Stout, J.C., 2009. The impacts of an invasive alien plant and its removal on native bees. Apidologie 40, 450–463.

Niu, H.B., Liu, W.X., Wan, F.H., Liu, B., 2007. An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. Plant Soil 294, 73–85.

Nobis, M., 2008. Invasive Neophyten auch im Wald? Wald Holz 8, 46–49.

Orr, S.P., Rudgers, J.A., Clay, K., 2005. Invasive plants can inhibit native tree seedlings: testing potential allelopathic mechanisms. Plant Ecol. 181, 153–165.

Pejchar, L., Mooney, H.A., 2009. Invasive species, ecosystem services and human well-being. Trends Ecol. Evol. 24, 497–504.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288.

R Development Core Team, 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reinhart, K.O., Callaway, R.M., 2006. Soil biota and invasive plants. New Phytol. 170, 445–457.

Richardson, D.M., Allsopp, N., D'Antonio, C.M., Milton, S.J., Rejmanek, M., 2000. Plant invasions – the role of mutualisms. Biol. Rev. 75, 65–93.

Roberts, K.J., Anderson, R.C., 2001. Effect of garlic mustard *Alliaria petiolata* (Beib. Cavara & Grande) extracts on plants and arbuscular mycorrhizal (AM) fungi. Am. Midl. Nat. 146, 146–152.

Rodgers, V.L., Wolfe, B.E., Werden, L.K., Finzi, A.C., 2008. The invasive species *Alliaria petiolata* (garlic mustard) increases soil nutrient availability in northern hardwood-conifer forests. Oecologia 157, 459–471.

Ruckli, R., Rusterholz, H.-P., Baur, B., 2013. Invasion of *Impatiens glandulifera* affects terrestrial gastropods by altering microclimate. Acta. Oecol. 47, 16–23.

Ruckli, R., Hesse, K., Gaetan, G., Rusterholz, H.-P., Baur, B., in press. Inhibitory potential of naphthoquinones leaching from leaves and exuding from roots of the invasive plant *Impatiens glandulifera*. J. Chem. Ecol. (2014).

Rusterholz, H.-P., Wirz, D., Baur, B., 2012. Garden waste deposits as a source for non-native plants in mixed deciduous forests. Appl. Veg. Sci. 15, 329–337.

Sanon, A., Beguiristain, T., Cebron, A., Berthelin, J., Ndoye, I., Leyval, C., Sylla, S., Duponnois, R., 2009. Changes in soil diversity and global activities following invasions of the exotic invasive plant, *Amaranthus viridis* L., decrease the growth of native sahelian *Acacia* species. FEMS Microbiol. Ecol. 70, 118–131.

Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Appl. Env. Microbiol. 43, 1256–1261.

Schroeder, M.S., Janos, D.P., 2005. Plant growth, phosphorus nutrition, and root morphological responses to arbuscular mycorrhizas, phosphorus fertilization, and intraspecific density. Mycorrhiza 15, 203–216.
Schussler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the glomeromycota: phylogeny and evolution. Mycol. Res. 105, 1413–1421.
Smith, S.E., Read, D., 2009. Mycorrhizal Symbiosis, third ed. Academic, Amsterdam.
Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.A., Soltanpour, P.N., Tabatabai, M.A., Jhonston, C.T., Sumner, M.E., 1996. Methods of Soil Analysis, Part 3, Chemical Methods, third ed. ASA, Madison.
Stinson, K.A., Campbell, S.A., Powell, J.R., Wolfe, B.E., Callaway, R.M., Thelen, G.C., Hallett, S.G., Prati, D., Klironomos, J.N., 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biol. 4, 727–731.
Suding, K.N., LeJeune, K.D., Seastedt, T.R., 2004. Competitive impacts and responses of an invasive weed: dependencies on nitrogen and phosphorus availability. Oecologia 141, 526–535.
Stoll, P., Gatzsch, K., Rusterholz, H.-P., Baur, B., 2012. Response of plant and gastropod species to knotweed invasion. Basic Appl. Ecol. 13, 232–240.
Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1, 301–307.
Tanner, R.A., Gange, A.C., 2013. The impact of two non-native plant species on native flora performance: potential implications for habitat restoration. Plant Ecol. 214, 423–432.
Thorpe, A.S., Archer, V., DeLuca, T.H., 2006. The invasive forb, *Centaurea maculosa*, increases phosphorus availability in Montana grasslands. Appl. Soil Ecol. 32, 118–122.
Titus, J.H., Leps, J., 2000. The response of arbuscular mycorrhizae to fertilization, mowing, and removal of dominant species in a diverse oligotrophic wet meadow. Am. J. Bot. 87, 392–401.
Vierheilig, H., Coughlan, A.P., Wyss, U., Piche, Y., 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl. Environ. Microbiol. 64, 5004–5007.
Wang, B., Qiu, Y.L., 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16, 299–363.
Weber, G., Claus, M., 2000. The influence of chemical soil factors on the development of VA mycorrhiza of ash (*Fraxinus excelsior* L.) and sycamore (*Acer pseudoplatanus* L.) in pot experiments. J. Plant Nutr. Soil Sci. 163, 609–616.
Weidenhamer, J.D., Callaway, R.M., 2010. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. J. Chem. Ecol. 36, 59–69.
Wolfe, B.E., Rodgers, V.L., Stinson, K.A., Pringle, A., 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. J. Ecol. 96, 777–783.

CHAPTER 3

Disrupting ectomycorrhizal symbiosis: Indirect effects of an invasive plant on growth and survival of beech saplings

Regina Ruckli, Hans-Peter Rusterholz and Bruno Baur

ABSTRACT

Invasive plants can disrupt the relationship between soil organisms and native plants which may result in altered ecosystem functions and/or reduced biodiversity. The ectomycorrhizal (EM) symbiosis is important for the growth and survival of the majority of tree species in boreal and temperate zones. We examined the effect of the annual invasive plant *Impatiens glandulifera* on the EM-association and performance of *Fagus sylvatica* saplings at three different levels of disturbance in a controlled field experiment. A total of 1188 one-year-old saplings with pruned roots were planted in plots invaded by *I. glandulifera*, in plots from which the invasive plant was manually removed and in plots which were not yet colonized by the invasive plant. The 54 (3 x 18) plots were equally distributed over three forest areas which were differently affected by a wind throw 8 years prior to the experiment. The saplings including the full root systems were harvested after 3, 6 and 15 months. A total of 54 EM morphotypes were recorded on the saplings' root tips in the different plots. The invasive plant reduced EM colonization of *F. sylvatica* saplings by 30% after 3 months and by 65% after 15 months. Furthermore, EM type richness was 32% lower after 15 months and both survival and biomass (root and shoot) of saplings were reduced by 18% and 30% in plots with *I. glandulifera*. Saplings growing in plots from which the invasive plant had been removed did not differ in EM colonization, number of EM morphotypes and in survival and biomass from saplings in uninvaded (control) plots. After 15 months, the EM morphotype communities on saplings in invaded plots differed from those in plots with removed invasive plants and in uninvaded plots. Former forest disturbance negatively affected EM colonization of *F. sylvatica* saplings but positively influenced their biomass. These findings demonstrate for the first time a negative impact of an annual invasive plant on the ectomycorrhizal symbiosis and performance of saplings of a forest tree.

Key words: *annual plant; disturbance; ectomycorrhiza;* Fagus sylvatica*; forest;* Impatiens glandulifera*; mycorrhizal symbiosis; non-native plant; plant-soil feedback.*

INTRODUCTION

The intentional and unintentional introduction of non-native species is considered as a major threat for the native biodiversity (Pimentel et al. 2005, Lambdon et al. 2008). Non-native species have the potential to affect an ecosystem seriously by changing species diversity, community structure and interactions between organisms, sometimes causing extinction of native species (Mooney and Hobbs 2000, Kourtev et al. 2002, Fitzpatrick et al. 2010). Environmental disturbance is usually assumed to change ecosystem properties providing opportunities for non-native species to invade a new area. Disturbance is considered as a key factor that facilitates the invasion of non-native species into various habitats (Elton 1958, D'Antonio et al. 1999, Hierro et al. 2006, Lockwood et al. 2007). Beside disturbance, other factors contribute to the success of non-native species in new areas. For example, there is growing evidence that mechanisms changing the composition and abundance of microbial soil organism increase the success of some invasive plant species (Richardson et al. 2000, Reinhart and Callaway 2006). Invasive plants are able to modify symbiotic interactions between microbial soil organisms and native host plants to their own advantage in the introduced habitats (Rudgers and Orr 2009, Weidenhamer and Callaway 2010).

The roots of many plant species develop intimate partnerships with particular soil fungi. Symbiosis with mycorrhizal fungi increases both the soil nutrient and water uptake of the host plant, strengthens pathogen resistance and protects the host plant during drought (Smith and Read 2008). The mycorrhizal symbiosis is a key factor determining the diversity of plant communities, their succession dynamics as well as the transport of resources within the ecosystem (Allen 1991, Smith and Read 2009, Johnson et al. 2012). Non-native plants invading natural communities have the potential to disrupt mycorrhizal symbiosis and/or alter the associated soil fungal community (Mummey and Rillig 2006, Wolfe and Klironomas 2008, Rudgers and Orr 2009). Some invasive species reduce the colonization rate of arbuscular mycorrhiza (AM) and/or of ectomycorrhiza (EM) in native plants (Roberts and Anderson 2001, Stinson et al. 2006, Wolfe and Klironomas 2008, Meinhardt and Gehring 2012). Furthermore, the infectivity and viability of native AM-spores can be inhibited by invasive plants (Roberts and Anderson 2001, Cantor et al. 2008).

EM-symbiosis is one of the key factors for tree growth and vigor influencing the forest dynamics (Smith and Read 2008). In disturbed areas, tree saplings maintain EM networks which are important for forest regeneration (Egli et al. 2001, Onguene and Kuyper 2002). However, there is little knowledge about the impact of invasive plants on the symbiosis

between saplings and associated EM-fungi. *Impatiens glandulifera* (Himalayan balsam), native in the western Himalaya, 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 Pysek 2006). In the last decade, *I. glandulifera* has increasingly invaded deciduous and coniferous forests disturbed by wind throws and/or intensive forest management (Nobis 2008). Relatively few studies have investigated the potential impact of *I. glandulifera* on native plant and animal communities. The presence of *I. glandulifera* causes slight changes in the cover of plant species and shifts in the species composition in riparian habitats (Maule et al. 2000, Hejda and Pysek 2006). Field studies showed that *I. glandulifera* competes successfully with native plants for pollinators, which could lead to a reduced plant fitness (Chittka and Schurkens 2001).

Here, we examined the potential impact of the invasive *I. glandulifera* on the symbiosis between *Fagus sylvatica* (European beech) saplings and EM fungi. *F. sylvatica* depends obligate on EM fungi. We designed a balanced field experiment with plots placed in patches of *I. glandulifera*, plots from which the invasive plant was regularly removed and plots which were not yet colonized by the invasive plant. The three plot types were equally distributed over three forest areas that were differently affected by a wind throw 8 years prior to the experiment, creating a natural gradient of disturbance. One-year-old non-mycorrhized *F. sylvatica* saplings planted in the experimental plots were harvested after 3, 6 and 15 months to assess *I. glandulifera*-induced changes in EM colonization, diversity of EM and survival and biomass of saplings. We tested the following hypotheses: (1) The invasion of *I. glandulifera* reduces both the EM colonization of fine roots of *F. sylvatica* saplings and the diversity of EM fungi. (2) The invasive plant-induced reduction of EM colonization increases with time. (3) The survival and biomass of *F. sylvatica* saplings are reduced in the presence of the invasive plant. (4) The former forest disturbance by the wind throw amplifies the impact of *I. glandulifera* on the symbiosis between *F. sylvatica* saplings and EM fungi.

MATERIALS AND METHODS

Study species

Impatiens glandulifera is an up to 2.5 m tall annual herb that produces up to 2500 seeds per individual (Beerling and Perrins 1993). The seeds are ejected from the fruits via ballochory and further dispersed by hydrochory and human activities. Seeds germinate later than the native vegetation in the following spring (DAISIE 2012).

Fagus sylvatica is the most abundant deciduous tree species in natural forest communities in Central Europe (Bolte et al. 2007). Seedlings and saplings of *F. sylvatica* are very shade-tolerant, but also adapted to high irradiation showing considerable phenotypic and ecophysiological plasticity (Parelle et al. 2006, Petritan et al. 2007).

Study system and field experiment

The experiment was carried out in three areas (each measuring 50 m x 180 m) in a mixed deciduous forest 15 km south of Basel, northern Switzerland (47°43' N, 7°55' E). In this region, the annual temperature averages 9.6 ^{o}C and the annual precipitation is 1021 mm (Meteo Swiss 2009). The three areas (situated within 1 km^2) were differently affected by the windstorm Lothar in 1999. Eight years after this storm, we assessed its disturbance intensity in the three areas by measuring the canopy closure of the remaining forest trees. One area with a canopy closure of 80% was considered as little disturbed, another area with a canopy cover of 50% as moderately disturbed, and the remaining area with a canopy closure of 2.5% as heavily disturbed (Appendix A). *Impatiens glandulifera* started to invade the forest in spring 2000.

In spring 2008 (8 years after invasion), we selected six homogenous patches of *I. glandulifera* in each area. In each patch situated 5–10 m apart from each other, we installed two 5 m x 5 m plots with similar *I. glandulifera* cover. One of the two plots was left invaded by *I. glandulifera* (hereafter referred to as invaded). In the other plot all *I. glandulifera* individuals were removed by hand (hereafter referred to as removed) every spring in the years 2008–2010. As an additional control, six 5 m x 5 m control plots that were not yet invaded by *I. glandulifera* were selected in close proximity (7–20 m) to the experimental plots in each area (referred to as uninvaded). This allows us to control for the slight mechanical disturbance of the soil by removing *I. glandulifera*. To prevent colonization of *I. glandulifera* in the not yet invaded plots, we removed all invasive plants growing in close proximity to these plots in 2009 and 2010. The additional control plots did not differ in soil characteristics (see below) and were colonized by the invasive plant in summer 2012 (3 years after the

initiation of the experiment). Thus, the experimental set-up consisted of 54 plots (18 invaded, 18 removed and 18 uninvaded) equally distributed over the three areas, which were differently affected by the storm.

1188 one-year-old *Fagus sylvatica* saplings obtained from a commercial forest nursery (Forstbaumschule Ingold AG; seed origin: Bützberg, Switzerland) were planted in the plots (22 saplings per plot) on April 18 and 19, 2009. To minimize potential edge effects the saplings were planted in an area of 1 m^2 at least 1 m apart from the plot border. Fine roots of the *F. sylvatica* saplings were pruned prior to planting to avoid transplanting EM from the nursery into the soil of the experimental plots. An effort was made to ensure that the height of the *F. sylvatica* saplings was similar in all plots (mean ± SE; invaded: 30.53 ± 0.61 cm, removed: 33.07 ± 0.84 cm, uninvaded: 31.41 ± 0.62 cm).

To evaluate any potential influence of soil characteristics on both EM colonization and biomass of *F. sylvatica* saplings, five randomly chosen soil samples were collected in each plot using a metal cylinder (soil depth: 5 cm; soil volume: 100 cm^3) in October 2009. The five soil samples of a plot were pooled, mixed and sieved (mesh width 2 mm). Soil water content (%) was determined using the fresh weight/dry weight ratio. Soil pH was measured in distilled water (1: 2.5 soil: water; Allen 1989). Soil organic matter content was assessed as loss on ignition of oven-dried soil at 700 ^oC for 24 h. Total soil organic nitrogen content was determined using the standard method of Kjeldahl (Allen 1989). Total phosphate was assessd using the molybdenum blue method following Allen (1989).

Data collection

To assess the effect of *I. glandulifera* on EM colonization and root and shoot biomass of *F. sylvatica* saplings, three saplings were harvested in each plot after 3 months (June 20, 2009), 6 months (September 20, 2009) and 15 months (June 21, 2010). Saplings with the entire root system were carefully dug out, put in plastic bags with soil of the corresponding plot and were kept in a climate chamber at 4 ^oC until examination of EM colonization. The number of viable and dead *F. sylvatica* saplings was recorded at each sampling date.

In the lab, roots were separated from shoots. The roots were washed free of soil and organic debris. Any adhering material was removed with forceps. For examining the EM colonization of each sapling, five living fine roots (diameter < 2 mm, length 4–5 cm) were randomly selected. In this subsample, the numbers of mycorrhized and non-mycorrhized root tips were counted using a binocular microscope (16 x magnification). Types of EM were determined using standard morphological characters, including color, shape and branching pattern (Agerer 1987–2002). Distinct EM morphotypes were described for further references (Appendix B). The dry weights of the saplings' shoots and coarse roots (> 2 mm) were

determined after oven drying (48 h, 60 ^{o}C) and that of the corresponding fine roots after lycophilization (48 h, -56 ^{o}C).

Statistical analyses

Statistical analyses were calculated using R 1.12.1 (R Development Core Team, 2010). To avoid spatial pseudoreplication, analyses were performed with the mean values of each plot. We primarily applied regression analyses to examine the relationships between EM colonization and fineroot biomass and between EM morphotypes and the fineroot biomass (EM colonization: R^2= 0.26, N = 162, P < 0.0001; EM morphotypes: R^2= 0.31, N = 162, P = 0.014). Linear mixed models for temporal pseudoreplication (LME) were used to analyse the effect of harvesting time, disturbance intensity, treatment, assessed soil characteristics, total biomass and biomass of *F. sylvatica* roots and shoots on the residuals of EM colonization and EM morphotypes. Harvesting time and treatment (invaded, removed, uninvaded) were included as fixed factors, plot nested in time as random factor, disturbance intensity, total biomass, biomass of *F. sylvatica* roots and shoots and soil characteristics as cofactors. The same structured LME was used to examine whether the invasion of *I. glandulifera* affects the Fisher's alpha diversity of EM morphotypes. To assess the effect of the invasive plant on survival of *F. sylvatica* saplings and biomass data, we used a similar LME as above, but added EM colonization rate and number of EM morphotypes as additional cofactors to the model. Harvesting time and treatment (invaded, removed, uninvaded) were included as fixed factors and plot nested in time as random factor. If necessary data were log or arsine-transformed to obtain normally distributed residuals. The linear mixed models were stepwise reduced as recommended by Crawley (2007).

Permutational multivariate analysis of variance (PERMANOVA; Anderson 2001, 2005, McArdle and Anderson 2001) was used to test whether the invasion of *I. glandulifera* affected the EM morphotype community. A preliminary analysis revealed that EM morphotype communities on *F. sylvatica* roots differed among the harvesting times. Therefore, data obtained after 3, 6 and 15 months were analyzed separately. In the analyses treatment (invaded, removed, uninvaded) was considered as fixed factor and disturbance intensity as cofactor. Differences in the structure of EM morphotype communities were calculated using Bray-Curtis dissimilarity index. Treatment effects were examined with a posteriori pairwise comparison using the PERMANOVA t-statistic. All PERMANOVA tests were based on 999 permutations of the untransformed raw data.

RESULTS

EM colonization of F. sylvatica saplings

Invasion of *I. glandulifera* resulted in a significant reduction of EM colonization on the fine roots of *F. sylvatica* saplings (Table 1, Fig. 1). This reduction increased with the time saplings were growing in *I. glandulifera* plots. After 3 months, EM colonization was 30% lower in invaded plots than both in plots from which *I. glandulifera* had been removed and in uninvaded plots (Table 1, Fig. 1). After 6 and 15 months, the reduction of EM colonization was 60% and 65%, respectively (Fig. 1). The significant treatment x harvesting time interaction indicates that EM colonization was differently reduced after 3, 6 and 15 months (Fig. 1). The LME-analyses revealed that EM colonization of saplings increased with increasing fine root biomass and that soil moisture did not directly affect EM colonization. However, soil moisture differently influenced EM colonization in the different treatments (Table 1).

Number of EM morphotypes on F. sylvatica saplings

A total of 54 EM morphotypes were found on roots of *F. sylvatica* saplings (Appendix B). Considering the different harvesting times, the number of EM morphotypes increased from 22 after 3 months to 52 after 15 months. Overall, the presence of *I. glandulifera* reduced the number of EM morphotypes (Fig. 2). Saplings growing in invaded plots harbored 15–43% fewer EM morphotypes than those growing in the plots with removed *I. glandulifera* and in uninvaded plots (Fig. 2). Fisher's alpha diversity of EM morphotypes was also reduced by the invasion of *I. glandulifera* (Table 1). Considering all plots, EM diversity decreased with time (2.47 ± 0.10 after 3 months, 2.25 ± 0.09 after 6 months, 1.62 ± 0.07 after 15 months; Table 2).

Considering the fine roots of single saplings, the number of EM morphotypes decreased from 6.2 ± 0.2 (mean ± SE) after 3 months, to 5.2 ± 0.2 after 6 months, and 4.4 ± 0.2 after 15 months. The significant treatment x harvesting time interaction indicates that the number of EM morphotypes in the three treatments was differently influenced after 3, 6 and 15 months. LME-analysis revealed that the number of EM morphotypes also increased with increasing fine root biomass (Table 1).

EM morphotype communities on F. sylvatica roots

The presence of *I. glandulifera* changed the composition of EM morphotypes in roots of *F. sylvatica* saplings (Table 3). This effect was more pronounced after 6 and 15 months than

after 3 months. Furthermore, the EM morphotype composition was influenced by the intensity of former forest disturbance (Table 3). Pairwise post hoc tests indicated that the EM morphotype composition after 6 and 15 months differed between saplings growing in invaded plots and those in plots with removed *I. glandulifera* as well as between saplings in invaded and uninvaded plots (Table 3). In contrast, saplings growing in uninvaded plots did not differ in EM morphotype composition from those in plots with removed *I. glandulifera* after 15 months (Table 3).

Survival and biomass of F. sylvatica saplings

The invasion of *I. glandulifera* reduced the survival of *F. sylvatica* saplings (Table 1). After 15 months, the survival rate of saplings growing in invaded plots was 18% lower than those of saplings growing in plots in which *I. glandulifera* had been removed and in uninvaded plots (Tables 1, 2). The time x treatment interaction indicates that survival of *F. sylvatica* saplings was differently affected by the different treatments.

The total biomass of *F. sylvatica* saplings and the biomass of their roots and shoots were reduced by 46–71% in plots invaded by *I. glandulifera* compared to those in plots from which the plant had been removed and in uninvaded plots (Table 4, Appendix C).

Effect of former forest disturbance

The intensity of former forest disturbance affected EM colonization. After 6 and 15 months, EM colonization on *F. sylvatica* saplings in the highly disturbed area was reduced by 11% and 21% compared to those in the little and moderately disturbed areas (Table 1, Fig. 1). Furthermore, the intensity of former forest disturbance negatively affected the number of EM morphotypes. Saplings growing in the highly disturbed area had 19–25% fewer EM morphotypes than those growing in the less disturbed forest areas (Table 1, Fig. 2). Fisher's alpha diversity of EM morphotypes was also reduced by the intensity of forest disturbance (Table 1). The lowest EM-diversity was found in the highly disturbed forest area (Table 2). Furthermore, sapling survival decreased with increasing level of forest disturbance (Tables 1, 2). Finally, sapling biomass and those of their roots and shoots increased with increasing intensity of former forest disturbance (Table 4, Appendix C).

DISCUSSION

Our results demonstrate that the invasive plant *Impatiens glandulifera* reduces the EM colonization and number of EM morphotypes and changes the composition of EM morphotypes on *Fagus sylvatica* saplings, which in turn have both a lower biomass and reduced survival rate compared to control saplings. The invasive plant-induced changes increased with time independent of the intensity of former forest disturbance.

There is increasing evidence indicating that invasive plants influence mycorrhizal associations of native trees. The reduction in EM colonization recorded in our study (up to 65%) is, however, somewhat higher than reported in the invasive *Alliaria petiolata* affecting *Pinus strobus* seedlings (reduction 40–50%; Wolfe et al. 2008), the native invasive *Kalmia angustifolia* influencing *Picea mariana* (40%; Yamasaki et al. 1998) and in the invasive *Tamarix* spp. on *Populus fremontii* (50%; Meinhardt and Gehring 2012). However, differences in study design, sampling schedule and plant life form should be taken into account when different studies on the effects of invasive species on EM association of tree species are compared. In contrast to our experimental approach with removal and control, the majority of studies are field surveys that either sampled the focal trees at different distances from the invasive plant or used soil root samples. *Impatiens glandulifera* is an annual species, while previous studies considered exclusively biennial and perennial invasive plants (Yamasaki et al. 1998, Wolfe et al. 2008, Meinhardt and Gehring 2012). The pronounced effect of *I. glandulifera* on EM colonization is therefore surprising. This could be explained by a species-specific response of *F. sylvatica* to the invasive plant. Moreover, experimental approaches as used in the present study rule out potential differences in plant performance and in historical and initial conditions between invaded and uninvaded plots. Controlled field experiments may therefore show more distinct results than field surveys disturbed by confounding factors (Hulme and Brenner 2006, Lopezaraiza-Mikel et al. 2007, Nienhuis et al. 2009).

The effect of *I. glandulifera* on EM colonization increased with progressive vegetation season. The low EM colonization of *F. sylvatica* saplings recorded in the initial phase of the experiment (after 3 months) might be a result of planting saplings with pruned roots. In plots with *I. glandulifera*, however, saplings harvested after 6 and 15 months had a significantly reduced EM colonization compared to those sampled after 3 months. After 6 months, *F. sylvatica* saplings growing in control plots without the invasive plant exhibited similar EM colonization rates as previously reported in young or mature *F. sylvatica* trees (Druebert et al. 2009, Goicoechea et al. 2009, Beniwal et al. 2011).

We found more than 50 EM morphotypes on *F. sylvatica* saplings with 4–6 types on single saplings. In other beech forests, 30–75 EM morphotypes have been recorded (Buee et al. 2005, De Roman et al. 2005, Grebenc and Kraigher 2007). Their number depended on forest and soil type, season and the particular method used for EM identification. Different EM morphotypes may vary in ecological function, e.g. in uptake of specific nutrients (Agerer 2001, Erland and Taylor 2002). A high diversity of EM morphotypes is therefore beneficial for the nutrient uptake, water supply and growth of numerous trees (Baxter and Dighton 2001, 2005). For example, biomass production of *Betula pendula* saplings was positively related to EM diversity under low fertility conditions (Jonsson et al. 2001).

To our knowledge, the potential influence of invasive plants on the EM morphotype richness of forest trees has so far not been examined. However, negative effects of invasive plants on both AM richness and colonization of trees have been reported. Mummey and Rillig (2006) found a decrease in AM diversity in native grasslands caused by the invasive plant *Centaurea maculosa*. Barto et al. (2011) demonstrated that *Alliaria petiolata* reduced the AM colonization on maple seedlings (*Acer saccharum*), but the invasive plant did not alter the AM richness.

Our study is the first demonstrating the negative effects of an invasive plant on EM colonization and EM diversity and changes in EM morphotype compositions on roots of a forest tree. The observed decline in EM morphotype diversity on *F. sylvatica* saplings in invaded plots might be a result of the reduced EM colonization rate, as indicated by the correlation analysis. A reduced number of EM morphotypes as well as a shift in the EM morphotype composition may have far reaching consequences for the nutrient and water supply of the saplings.

Fagus sylvatica saplings growing in plots from which *I. glandulifera* was regularly removed showed similar EM colonization rates and EM diversity as those saplings growing in uninvaded control plots (Fig. 1). This suggests that allelopathic effects of *I. glandulifera* could inhibit EM symbiosis. Indeed, EM fungi were found to react sensitively to allelochemical active compounds detected in leaf litter, plants and roots (Rice 1979, Rose et al. 1983, Cote and Thibault 1988). Several studies demonstrated allelopathic effects of invasive plants to be the key factor for the disruption of AM (Roberts and Anderson 2001, Callaway et al. 2004, Bais et al. 2006, Mummey and Rillig 2006) and EM symbiosis (Wolfe et al. 2008). *Impatiens glandulifera* contains secondary plant compounds with antifungal effects (Lobstein et al. 2001) indicating that allelopathic effects disturb the EM symbiosis in *F. sylvaticus.*

EM symbiosis facilitates the uptake of soil phosphorus and nitrogen in plants (Smith and Read 2008). Weidenhamer and Callaway (2010) showed that invasive plants are able to modify soil nutrient composition. In our study, however, we recorded similar P and N

concentrations in the topsoil of all treatments. Thus, the observed reduction in EM colonization on *F. sylvatica* saplings was not a result of different soil nutrient concentrations.

Soil moisture content is another important factor determining the abundance of single EM morphotypes (Shi et al. 2002). In our experiment, soil moisture was 12–18% higher in invaded plots than in both types of control plots during the period of *I. glandulifera* growth (data not shown). However, the number of EM morphotypes was not affected by soil moisture.

Light availability is another crucial factor for the development of EM associations (Zelenznik et al. 2007, Druebert et al. 2009). We considered the density of *I. glandulifera* as a surrogate for light availability. Correlation analyses revealed that the density of *I. glandulifera* had no influence on the EM colonization on *F. sylvatica* saplings, indicating that differences in light availability between the treatments were not causing the reduction in EM colonization.

Fagus sylvatica saplings in invaded plots had not only a reduced EM colonization but also a lower survival rate than those growing in control plots. Saplings with a low EM colonization might be more vulnerable to pathogen infection resulting in a reduced survivorship (Smith and Read 2008). Interestingly, the biomass of saplings was also reduced in invaded plots, but the saplings' survival rate was not related to their biomass. In contrast, Ammer et al. (2011) reported that *I. glandulifera* did not affect growth and survival of *Abies alba* und *Pinus abies* seedlings. This discrepancy could be due to the saplings' species-specific response to the invasive plant.

Forest disturbances can affect both EM colonization and EM type composition in tree species (Egli 2001, Jones 2003). In the present study, the former windstorm influenced the EM association of *F. sylvatica* saplings. EM colonization and the number of EM morphotypes decreased with increasing intensity of forest disturbance. The survival rate of the saplings was also negatively affected by the intensity of disturbance. In contrast, the larger biomass of saplings found in the most disturbed forest areas might be a result of the more favorable light conditions for plant growth compared to those of more shady plants in less disturbed forest areas.

Although the impact of *I. glandulifera* on native plant species seemed to be less severe than that of other invasive species (Hejda and Pysek 2009, Ammer et al. 2011), we provide experimental evidence that this invasive plant reduces the survival of *F. sylvatica* saplings by negatively affecting their important EM symbiosis. This effect was similar in the differently disturbed forest areas indicating that the reduction of EM symbiosis on *F. sylvatica* saplings by *I. glandulifera* might be a widespread phenomenon. The spread of *I. glandulifera* in mixed deciduous forests is therefore a serious threat to forest regeneration. Several other tree

species are connected to the same EM network as *F. sylvatica* (Lang et al. 2011). Thus, further tree species could be negatively affected by *I. glandulifera*. In the longterm this may result in high economic losses for forestry.

ACKNOWLEDGMENTS

We thank Sietse van der Linde and Anette Baur for comments on the manuscript, Armando Lenz, Jonas Küng, Esther Lischer, Miriam Mühlebach, Maya Schefer and Viola Ruckli for field assistance and Simon Egli and Silvia Hutter for the introduction into EM fungi determination.

LITERATURE CITED

Agerer, R. 1987-2002. Colour atlas of ectomycorrhizae. München, Einhorn Verlag, Germany.

Agerer, R. 2001. Exploration types of ectomycorrhizae - A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza **11**:107-114.

Allen, M. F. 1991. The ecology of mycorrhizae. Cambridge, University Press, New York, USA.

Allen, S. E. 1989. Chemical analysis of ecological materials. 2nd edition. Blackwell Scientific Publications, Oxford, UK.

Ammer, C., P. Schall, R. Woerdehoff, K. Lamatsch, and M. Bachmann. 2011. Does tree seedling growth and survival require weeding of Himalayan balsam (*Impatiens glandulifera*)? European Journal of Forest Research **130**:107-116.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology **26**: 32-46.

Anderson, M. J. 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, New Zealand.

Bais, H. P., T. L. Weir, L. G. Perry, S. Gilroy, and J. M. Vivanco. 2006. The role of root exudates in rhizosphere interations with plants and other organisms. Annual Review of Plant Biology **57**:233-266.

Barto, E. K., P. M. Antunes, K. Stinson, A. M. Koch, J. N. Klironomos, and D. Cipollini. 2011. Differences in arbuscular mycorrhizal fungal communities associated with sugar maple seedlings in and outside of invaded garlic mustard forest patches. Biological Invasions **13**:1043-1055.

Baxter, J. W., and J. Digthon. 2001. Ectomycorrhizal diversity alters growth and nutrient acquisition of grey brich (*Betula populifolia*) seedlings in host-symbiont culture conditions. New Phytologist **152:**13-149.

Baxter, J. W., and J. Digthon. 2005. Diversity-functioning relationships in ectomycorrhizal fungal communities. Pages 383-398 *in* J. Digthon, J. F. White and P. Oudemans, editors. The fungal community: Its organization and role in the ecosystem. 3rd edtition. Marcel-Dekker, New York, USA.

Beerling, D. J., and J. M. Perrins. 1993. *Impatiens glandulifera* Royle (*Impatiens roylei* Walp.). Journal of Ecology **81**:367-382.

Beniwal, R. S., M. S. Hooda, and A. Polle. 2011. Amelioration of planting stress by soil amendment with a hydrogel-mycorrhiza mixture for early establishment of beech (*Fagus sylvatica* L.) seedlings. Annuals of Forest Science **68**:803-810.

Bolte, A., T. Czajkowski, and T. Kompa. 2007. The north-eastern distribution range of European beech - a review. Forestry **80**:413-429.

Buee, M., D. Vairelles, and J. Garbaye. 2005. Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus sylvatica*) forest subjected to two thinning regimes. Mycorrhiza **15**:235-245.

Callaway, R. M., G. C. Thelen, S. Barth, P. W. Ramsey, and J. E. Gannon. 2004. Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. Ecology **85**:1062-1071.

Cantor, A., A. Hale, J. Aaron, M. B. Traw, and S. Kalisz. 2011. Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. Biological Invasions **13**:3015-3025.

Chittka, L., and S. Schurkens. 2001. Successful invasion of a floral market - An exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature **411**:653-653.

Cote, J. F., and J. R. Thibault. 1988. Allelopathic potential of raspberry folliar leachates on growth of ectomycorrhizal fungi associated with black spruce. American Journal of Botany **75**:966-970.

Crawley, M. J., 2007. The R book. Wiley, Chichester, England.

D'Antonio, C. M., T. L. Dudley, and M. Mack. 1999. Disturbance and biological invasions: direct effects and feedbacks. Pages 413-452 *in* L. R. Walker, editors. Ecosystems of disturbed ground. Elsevier, Amsterdam, Netherlands.

DAISIE European Invasive Alien Species Gateway, 2012. *Impatiens glandulifera*. http://www.europe-aliens.org/speciesFactsheet.do?speciesId=17367. (accessed 01.04.12)

De Roman, M., V. Claveria, and A. M. De Miguel. 2005. A revision of the descriptions of ectomycorrhizas published since 1961. Mycological Research **109**:1063-1104.

Druebert, C., C. Lang, K. Valtanen, and A. Polle. 2009. Beech carbon productivity as driver of ectomycorrhizal abundance and diversity. Plant Cell and Environment **32**:992-1003.

Egli, S., M. Peter, and S., Falcato. 2002. Dynamics of ectomycorrhizal fungi after windthrow. Forest Snow and Landscape Research **77**:81-88.

Elton C. S. 1958. The ecology of invasions by animals and plants. Methuen, London, UK.

Erland, S., and A. F. S. Talyor. 2002. Diversity of ectomycorrhizal fungal communities in relation to the abiotic environment. Pages 163-200 *in*: van der Heijden, M. G. A., I. Sanders (Eds.), Mycorrhizal ecology. Ecological Studies. Springer, Berlin, Heidelberg, Germany.

Fitzpatrick, B. M., J. R. Johnson, D. K. Kump, J. J. Smith, S. R. Voss, and H. B. Shaffer. 2010. Rapid spread of invasive genes into a threatened native species. Proceedings of the National Academy of Sciences of the United States of America **107**:3606-3610.

Goicoechea, N., I. Closa, and A. Maria de Miguel. 2009. Ectomycorrhizal communities within beech (*Fagus sylvatica* L.) forests that naturally regenerate from clear-cutting in northern Spain. New Forests **38**:157-175.

Grebenc, T., and H. Kraigher. 2007. Types of ectomycorrhiza of mature beech and spruce at ozone-fumigated and control forest plots. Environmental Monitoring and Assessment **128**:47-59.

Hejda, M., and P. Pysek. 2006.What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? Biological Conservation **132**:143-152.

Hejda, M., P. Pysek, and V. Jarosik. 2009. Impact of invasive plants on the species richness, diversity and composition of invaded communities. Journal of Ecology **97**:393-403.

Hierro, H. L., D. Villarreal, Ö. Eren, J. M. Graham, and R. M. Callaway. 2006. Disturbance facilitates invasions: The effects are stronger abroad than at home. American Naturalist **168**:144-156.

Hulme, P. E., and E. T. Bremner. 2006. Assessing the impact of *Impatiens glandulifera* on riparian habitats: partitioning diversity components following species removal. Journal of Applied Ecology **43**:43-50.

Jones, M. D., D. M. Durall, and J. W. G. Cairney. 2003. Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytologist **157**: 399-422.

Johnsson, N. C., M. Nilsson D. A. Wardle, and O. Zackrisson. 2001. Context dependent effects of ectomycorrhizals species richness on tree seedling productivity. Oikos **93**:353-364.

Johnson, D., F. Martin, J. W. G. Cairney, and I. C. Anderson. 2012. The importance of individuals: interspecific diversity of mycorrhizal plants and fungi in ecosystems. New Phytologist **194**:614-628.

Kourtev, P. S., J. G. Ehrenfeld, and M. Haggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology **83**:3152-3166.

Kraigher, H., S. A. S. Petkovsek, T. Grebenc, and P. Simoncic. 2007. Types of ectomycorrhiza as pollution stress indicators: Case studies in Slovenia. Environmental Monitoring and Assessment **128**:31-45.
Lambdon, P. W., P. Pysek, C. Basnou, M. Hejda, M. Arianoutsou, F. Essl, V. Jarosik, J. Pergl, M. Winter, P. Anastasiu, P. Andriopoulos, I. Bazos, G. Brundu, L. Celesti-Grapow, P. Chassot, P. Delipetrou, M. Josefsson, S. Kark, S. Klotz, Y. Kokkoris, I. Kuehn, H. Marchante, I. Perglova, J. Pino, M. Vila, A. Zikos, D. Roy, and P. E. Hulme. 2008. Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs. Preslia **80**:101-149.
Lang, C., J. Seven, and A. Polle. 2011. Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. Mycorrhiza **21**:297-308.
Lobstein, A., X. Brenne, E. Feist, N. Metz, B. Weniger, and R. Anton. 2001. Quantitative determination of naphthoquinones of *Impatiens* species. Phytochemical Analysis **12**:202-205.
Lookwood, J. L., M. P. Hoopes, and M. P. Marchetti. 2007. Invasion ecology. Blackwell, Oxford.
Lopezaraiza-Mikel, M. E., R. B. Hayes, M. R. Whalley, and J. Memmott. 2007. The impact of an alien plant on a native plant-pollinator network: an experimental approach. Ecology Letters **10**:539-550.
Maule, H., M. Andrews, Watson C., and A. J. Cherrill. 2000. Distribution, biomass and effect on native species of *Impatiens glandulifera* in a deciduous woodland in northeast England. Aspects of Applied Biolology **58**:31-38.
McArdle, B. H., and Anderson, M. J. 2001. Fitting multivariate models to community data: a comment on distance- based redundancy analysis. Ecology **82**: 290-297.
Meinhardt, K. A., and C. A. Gehring. 2012. Disrupting mycorrhizal mutualisms: a potential mechanism by which exotic tamarisk outcompetes native cottonwoods. Ecological Applications **22**:532-549.
Meteo Swiss, 2009. Normwerte 1961–90. Federal Office of Meteorology and Climatology MeteoSwiss. http://www.meteoschweiz.ch. (accessed 10.11.10).
Mooney, H. A., and R. J. Hobbs. 2000. Invasive species in a changing world. Washington, DC, Island.
Mummey, D. L., and M. C. Rillig. 2006. The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil **288**:81-90.
Nienhuis, C. M., A. C. Dietzsch, and J. C. Stout. 2009. The impacts of an invasive alien plant and its removal on native bees. Apidologie **40**:450-463.
Nobis, M. 2008. Invasive Neophyten auch im Wald? Wald und Holz **8**:46-49.
Onguene N., and T. Kuyper. 2002. Importance of the ectomycorrhizal network for seedling survival and ectomycorrhiza formation in rain forest of south Cameroon. Mycorrhiza **12**:13-17.
Parelle, J., J. P. Roudaut, and M. Ducrey. 2006. Light acclimation and photosynthetic response of beech (*Fagus sylvatica* L.) saplings under artificial shading or natural Mediterranean conditions. Annals of Forest Science **63**:257-266.
Petritan, A. M., B. von Lüpke, and I. C. Petritan. 2007. Effects of shade on and mortality of maple (*Acer pseudoplatanus*), ash (*Fraxinus excelsoir*) and beech (*Fagus sylvatica*). Forestry **80**:397-412.
Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics **52**:273-288.
R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria,
Reinhart, K. O., and R. M. Callaway. 2006. Soil biota and invasive plants. New Phytologist **170**:445-457.

Rice, E. L. 1979. Allelopathy: an update. Botanical Review **45**:15-109.
Richardson, D. M., N. Allsopp, C. M. D'Antonio, S. J. Milton, and M. Rejmanek. 2000. Plant invasions - the role of mutualisms. Biological Reviews **75**:65-93.
Roberts, K. J., and R. C. Anderson. 2001. Effect of garlic mustard *Alliaria petiolata* (Beib. Cavara & Grande) extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist **146**:146-152.
Rose, S. L., D. A. Perry, D. Pilz, and M. M. Schoeneberger. 1983. Allelopathic effects of litter on the growth and colonization of mycorrhizal fungi. Journal of Chemical Ecology **9**:1153-1162.
Rudgers, J. A., and S. Orr. 2009. Non-native grass alters growth of native tree species via leaf and soil microbes. Journal of Ecology **97**:247-255.
Shi, L., M. Guttenberger, I. Kottke, and R. Hampp. 2002. The effect of drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure, and content of carbohydrates and nitrogen storage bodies of the fungi. Mycorrhiza **12**:303-311
Smith, S. E., and D. J. Read. 2008. Mycorrhizal symbiosis. 3rd edition. Academic, Amsterdam.
Stinson, K. A., S. A. Campbell, J. R. Powell, B. E. Wolfe, R. M. Callaway, G. C. Thelen, S. G. Hallett, D. Prati, and J. N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. Plos Biology **4**:727-731.
Weidenhamer, J. D., and R. M. Callaway. 2010. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. Journal of Chemical Ecology **36**:59-69.
Wolfe, B. E., V. L. Rodgers, K. A. Stinson, and A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. Journal of Ecology **96**:777-783.
Yamasaki, S. H., J. W. Fyles, K. N. Egger, and B. D. Titus. 1998. The effect of *Kalmia angustifolia* on the growth, nutrition, and ectomycorrhizal symbiont community of black spruce. Forest Ecology and Management **105**:197-207.
Zeleznik, P., M. Hrenko, C. Then, N. Koch, T. Grebenc, T. Levanic, and H. Kraigher. 2007. CASIROZ: Root parameters and types of ectomycorrhiza of young beech plants exposed to different ozone and light regimes. Plant Biology **9**:298-308.

TABLES

TABLE 1. Summary of the LME analyses on EM colonization, numbers of EM morphotypes, Fisher's alpha diversity of EM and survival of *F. sylvatica* saplings.

	EM colonization (%)			Number of EM morphotypes			Fisher 's alpha			Survival of *F. sylvatica* (%)		
	df	F	P	df	F	P	df	F	P	df	F	P
Treatment	2,131	101.15	<0.0001	2, 134	102.23	<.0001	2,134	14.19	<.0001	2,132	20.80	<.0001
Intensity of disturbance	1,131	61.51	0.0001	1, 134	62.17	<.0001	1,134	43.08	<.0001	1,132	6.36	0.0128
Harvesting time	2,10	23.62	0.0015	2, 10	23.52	0.0002	2,10	35.28	<.0001	2,10	12.39	0.0020
EM colonization (%)	†	†	†	†	†	†	†	†	†	1,132	15.678	0.0001
Number of Types of EM	†	†	†	†	†	†	†	†	†	–	–	–
Fineroot biomass (mg)	1,131	7.78	0.0097	1,101	7.54	0.0068	1,134	3.47	0.0647	–	–	–
Soil moisture (%)	1,131	0.49	0.4524	–	–	–	–	–	–	–	–	–
Total organic matter (%)	–	–	–	–	–	–	–	–	–	1,133	3.933	0.0494
Intensity of disturbance x treatment	1,131	0.88	0.1442	4,134	2.31	0.1028	2,134	4.41	0.0140	4,133	2.382	0.0963
Harvesting time x treatment	4,131	9.00	<0.0001	4,134	5.83	0.0002	4,134	0.87	0.4792	4,133	2.654	0.0358
Soil moisture x treatment	2,131	3.24	0.0420	–	–	–	–	–	–	–	–	–

Notes: – cofactor was excluded from the model
† never included in the model

TABLE 2. Mean ± SE of Fisher's alpha diversity of EM and survival of *F. sylvatica* saplings growing in the different treatments (invaded, removed, uninvaded) in the differently disturbed forest areas at the three harvesting times (n = 6, in each case).

Harvesting time	Intensity of Disturbance								
	little			moderate			high		
	invaded	removed	uninvaded	invaded	removed	uninvaded	invaded	removed	uninvaded
After 3 months									
Fisher's alpha	2.24 ± 0.27	3.32 ± 0.22	2.90 ± 0.19	2.41 ± 0.26	2.40 ± 0.27	3.06 ± 0.33	1.87 ± 0.07	1.60 ± 0.11	2.46 ± 0.29
F. sylvatica survival (%)	73.48 ± 1.40	75.76 ± 6.91	84.85 ± 3.65	73.48 ± 1.40	75.76 ± 6.91	84.85 ± 3.65	73.48 ± 1.40	75.76 ± 6.91	84.85 ± 3.65
After 6 months									
Fisher's alpha	1.84 ± 0.08	2.83 ± 0.12	2.84 ± 0.15	2.17 ± 0.29	2.59 ± 0.31	2.61 ± 0.26	1.48 ± 0.30	1.67 ± 0.01	2.18 ± 0.31
F. sylvatica survival (%)	71.97 ± 1.82	74.24 ± 7.58	81.82 ± 4.84	66.67 ± 5.46	90.15 ± 3.79	78.03 ± 6.15	60.61 ± 2.54	65.15 ± 5.46	78.79 ± 3.83
After 15 months									
Fisher's alpha	1.54 ± 0.14	1.95 ± 0.16	1.97 ± 0.27	1.44 ± 0.17	1.86 ± 0.12	1.67 ± 0.30	1.26 ± 0.22	1.41 ± 0.11	1.50 ± 0.16
F. sylvatica survival (%)	62.12 ± 3.46	70.45 ± 6.51	67.42 ± 4.61	59.09 ± 4.39	81.81 ± 5.63	75.76 ± 5.71	53.03 ± 9.80	64.39 ± 5.30	70.45 ± 6.29

TABLE 3. Summary of the PERMANOVAs testing the effects of intensity of disturbance and treatment (invaded, removed, uninvaded) on the composition of EM morphotypes on *F. sylvatica* root tips after 3, 6 and 15 months and pairwise post hoc tests between treatments.

PERMANOVA		3 months		6 months		15 months	
Source	df	F	P	F	P	F	P
Treatment	2	1.54	0.0230	2.31	0.0010	2.50	0.0010
Intensity of disturbance	1	5.90	0.0010	2.55	0.0010	2.24	0.0030
Residual	50						
Total	53						
Pairwise post hoc tests between treatments		t	P	t	P	t	P
removed - invaded		1.28	0.0780	1.58	0.0010	1.63	0.0010
uninvaded - invaded		1.19	0.1230	1.63	0.0010	1.88	0.0010
uninvaded - removed		1.26	0.0540	1.33	0.0170	1.13	0.1700

TABLE 4. Summary of LME analyses on various aspects of biomass, the proportions of main root/fine root and shoot/root of *F. sylvatica* saplings.

		Total biomass		Shoot biomass		Root biomass		Mainroot		Fineroot		Mainroot:fineroot		Root:shoot		
	df	F	P	F	P	F	P	F	P	F	P	F	P	df	F	P
Treatment	2,134	18.28	<.0001	15.88	<.0001	17.39	<.0001	13.11	<.0001	28.59	<.0001	12.51	0.001	2,133	1.58	0.2108
Intensity of disturbance (Id)	1,134	45.56	<.0001	25.70	<.0001	57.16	<.0001	59.69	<.0001	13.16	0.0004	2.28	0.133	1,133	17.86	<.0001
Harvesting time	2,10	18.14	0.0005	20.95	0.0003	14.26	0.0012	5.03	0.0308	98.03	<.0001	63.37	<.0001	2,10	13.32	0.0015
EM colonization (%)	1,134	3.62	0.059	2.75	0.0996	–	–	3.88	0.0509	20.25	<.0001	15.57	0.0001	–	–	–
Types of EM	–	–	–	–	–	3.66	0.0579	–	–	–	–	–	–	–	–	–
Total phosphor	–	–	–	–	–	–	–	–	–	–	–	–	–	1,133	6.54	0.0117
Total organic matter (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	1,133	4.18	0.0429
Id:treatment	2,134	0.20	0.822	0.07	0.9313	0.59	0.5548	0.38	0.6846	3.65	0.0287	3.84	0.0219	2,134	2.65	0.0747
Harvesting time x treatment	4,134	1.24	0.298	1.79	0.1347	0.74	0.5664	0.62	0.6489	1.98	0.1010	4.465	0.0020	4,134	1.37	0.2480

Notes: – cofactor was excluded from the model

FIGURE LEGENDS

FIG. 1. EM colonization (%) of *Fagus sylvatica* saplings growing in plots invaded by *Impatiens glandulifera*, in plots from which the invasive plant had been removed and in uninvaded plots. The different plots were equally distributed in three differently disturbed forest areas. Saplings were harvested after 3 months (July 2009), 6 months (September 2009) and 15 months (July 2010). Mean values ± SE of six replicates are shown. Different letters indicate significant differences among the treatments (invaded, removed, uninvaded) within each forest area and for each harvesting time.

FIG. 2. Number of EM morphotypes on the roots of *Fagus sylvatica* saplings growing in plots invaded by *Impatiens glandulifera*, in plots from which the invasive plant had been removed and in uninvaded plots. The different plots were equally distributed in three differently disturbed forest areas. Saplings were harvested after 3 months (July 2009), 6 months (September 2009) and 15 months (July 2010). Mean values ± SE of six replicates are shown. Different letters indicate significant differences among the treatments (invaded, removed, uninvaded) within each forest area and for each harvesting time.

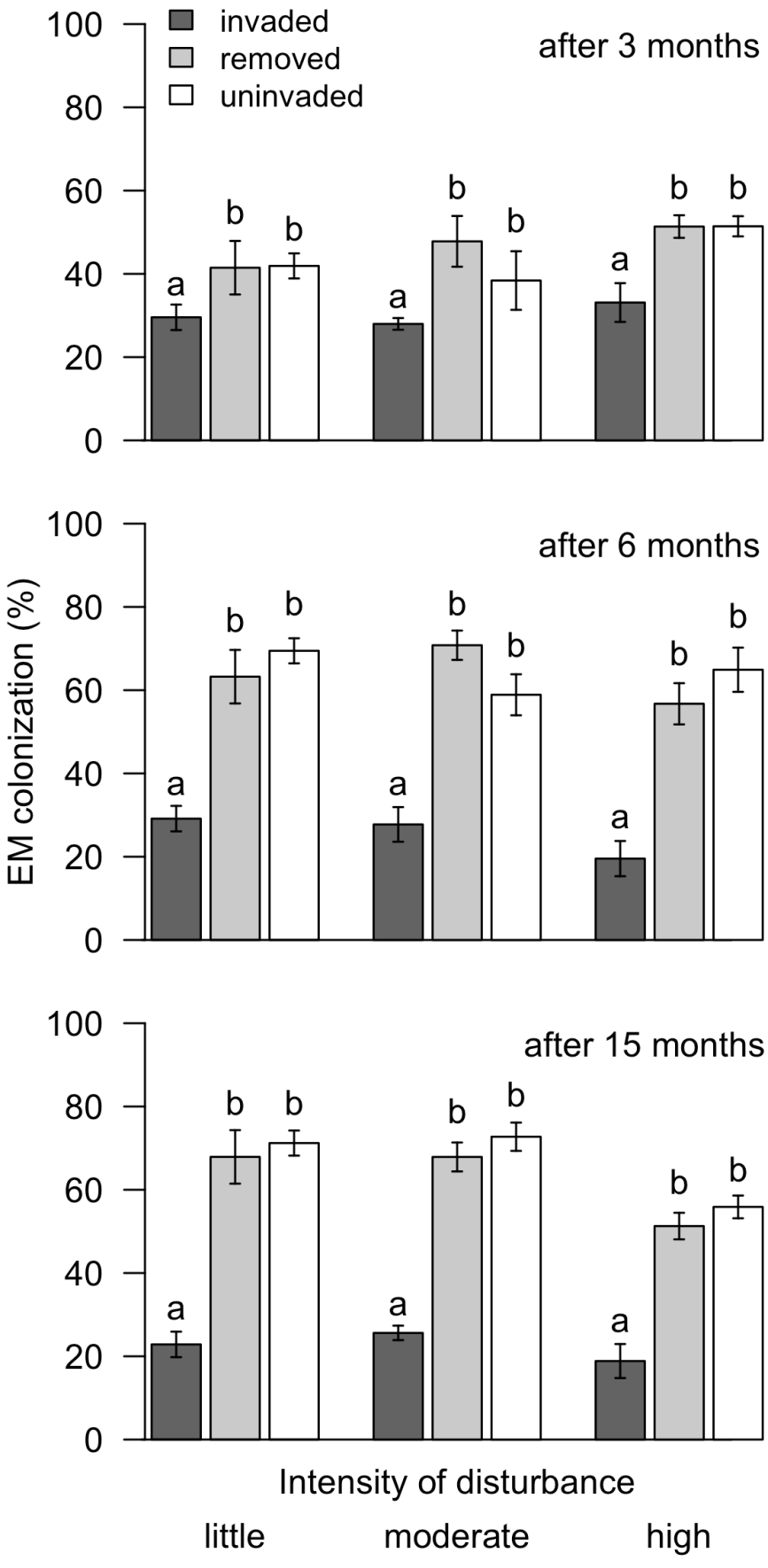
invaded
removed
uninvaded
after 3 months
after 6 months
after 15 months
EM colonization (%)
0
20
40
60
80
100
a
b
Intensity of disturbance
little
moderate
high

FIGURE 1

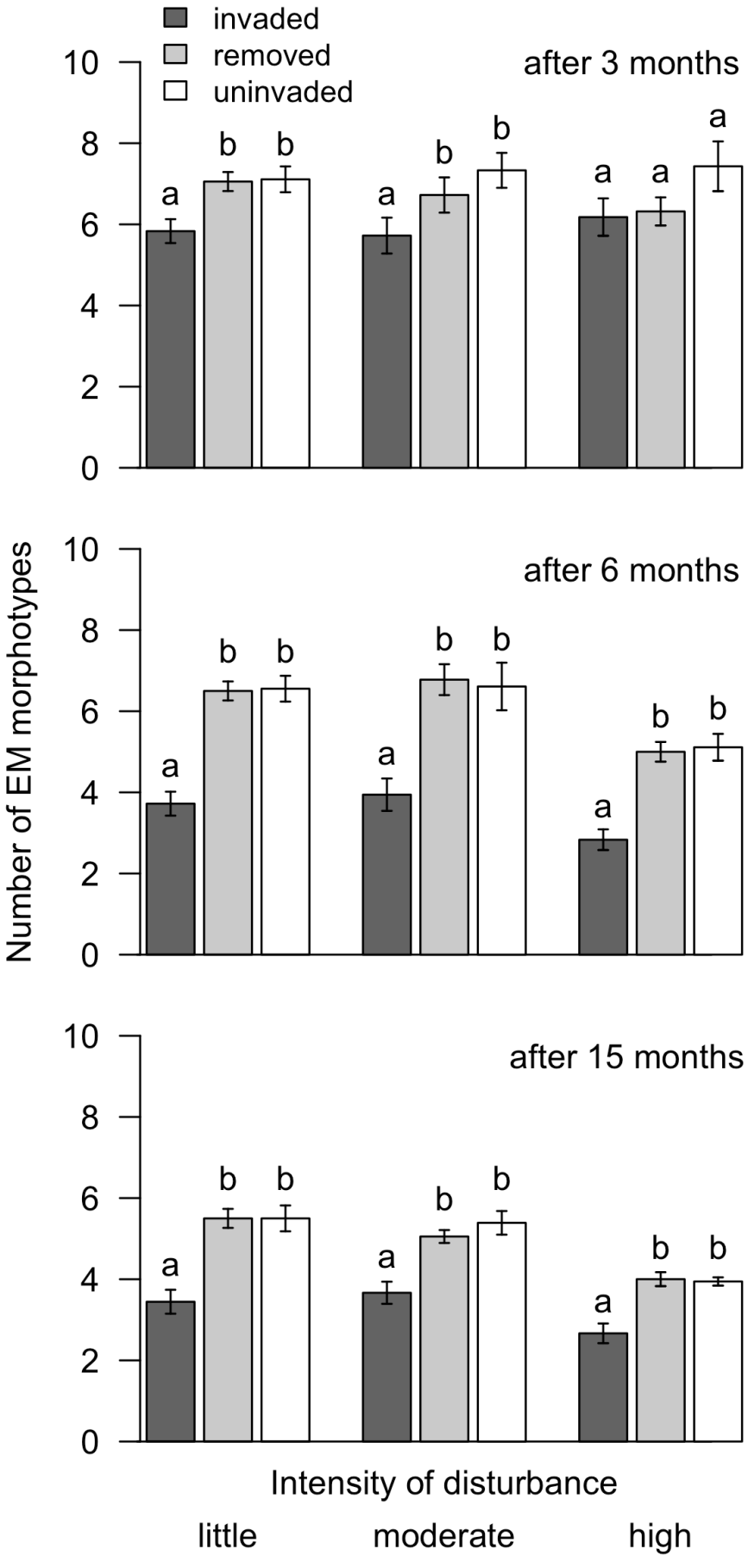
invaded
removed
uninvaded
after 3 months
after 6 months
after 15 months
Number of EM morphotypes
0
2
4
6
8
10
a
b
b
a
b
b
a
a
a
a
b
b
a
b
b
a
b
b
a
b
b
a
b
b
Intensity of disturbance
little
moderate
high

FIGURE 2

SUPPLEMENTAL MATERIAL

APPENDIX A

Characteristics of the three study areas which were differently disturbed by a windstorm 8 years prior to the present study.

	Intensity of disturbance by windstorm			
	little	moderate	high	P
Canopy closure (%) [a]	80 (50–80)	50 (50–70)	2.5 (0–10)	
Forest vegetation type [b]	Galio oderati-Fagetum luzuletosum	Luzulo sylvaticae-Fagetum typicum	Galio oderati-Fagetum luzuletosum	
Soil type [c]	Eutric haplic luvisol	Haplic luvisol	Gleyic cambisol	
Ground plant cover (%)	73 ± 4	68 ± 5	83 ± 3	0.046
Cover of *I. glandulifera* (%)[d]	85 ± 11	93 ± 7	82 ± 13	0.186
Biomass of *I. glandulifera* (dry weight, gm^{-2})[d]	237.9 ± 58.1	243.7 ± 54.9	118.9 ± 28.8	0.085
Soil pH	4.7 ± 0.04	4.6 ± 0.07	4.7 ± 0.05	0.799
Soil moisture (%)	21.2 ± 0.8	25.7 ± 1.1	31.6 ± 0.9	<0.0001
Total soil organic matter (%)	12.8 ± 0.6	16.9 ± 1.1	18.0 ± 1.0	<0.001
Total phosphorus (µg $PSO_4^- g^{-1}$ soil)	109.6 ± 4.6	161.3 ± 5.4	142.3 ± 3.7	<0.0001
Total organic nitrogen (%)	0.24 ± 0.01	0.33 ± 0.01	0.35 ± 0.01	<0.0001

Notes: Mean values ± SE, n = 18 plots for each intensity of disturbance
P-values resulting from one-way ANOVA or Kruskal-Wallis-test indicating differences between study areas

[a] Median and range
[b] Burnand and Hasspacher (1999)
[c] Walthert et al. (2004)
[d] n = 6

APPENDIX B

Morphological description of the ectomycorrhizal (EM) types on *Fagus sylvatica* saplings. A total of 1188 saplings were examined.

EM type	Color	Shape and size of mycorrhiza	Surface of mantle	Emanating hyphae	Rhizomorphs
1	Whitish	Unramified, straight, 1-2 x 0.25 mm	Rough	Not specifically distributed	n.o.
2	Light orange	Monopodial-pinnate, bent, 1 x 0.25 mm	Smooth	n.o.	n.o.
3	Greyish	Unramified, straight, 0.8 x 0.2 mm	Shiny	n.o.	n.o.
4	Reddish	Pyramidal-pinnate, straight, 2 x 1.5 mm	Milky covered with soil particles	Concentrated proximally	n.o.
5	Ochre with white ends	Monopodial-pinnate, straight, 1.5-2.5 x 0.3 mm	Smooth	n.o.	n.o.
6	Brown	Unramified, clubbed, 0.8 x 0.2 mm	Densely woolly	Branched, concentrated distally	n.o.
7	Brown	Unramified, straight, 0.8 x 0.4 mm	Loosely woolly, silvery	Not specifically distributed	Dark, round, infrequently
8	Dark	Unramified, sinuous, 1 x 0.3 mm	Rough	Not specifically distributed	Dark, round, infrequently
9	Orange	Unramified, straight, 1.5 x 0.4 mm	Milky	Lacking only at the tip	White, fan-like, infrequently
10	Violet	Irregular pinnate, bent	Smooth	n.o	n.o.
11	White	Unramified, round, 0.2 x 0.2 mm	Smooth	n.o	n.o.
12	White	Unramified, straight, 0.8 x 0.4 mm	Shiny, milky,	Spiny, not specifically distributed	n.o.
13	Brown	Irregular pinnate, bent, 1.2 x 0.2 mm	Densely woolly, , silvery	Not specifically distributed	Dark, flat, infrequently
14	Reddish	Monopodial-pyramidal, straight, 2.5 x 0.5 mm	Densely cottony, silvery	Not specifically distributed	White, round, concentrated proximally
15	Gold-brown with dark brown spots	Irregular-pinnate, straight, 1.5 x 0.5 mm	Shiny	Not specifically distributed	n.o.
16	Red-orange	Unramified, straight, 2-4 x 0.5 mm	Glistering	n.o.	n.o.
17	Dark brown-violet	Irregular-pinnate, slightly bent, 1-0.2 mm	Smooth, shiny	n.o.	n.o.

18	Ochre-pink	Irregular-pinnate, straight, 1.2 x 0.3 mm	Densely woolly	Concentrated proximally	n.o.
19	Yellow-orange	Unramified, straight, 3 x 0.5 mm	Smooth	n.o.	n.o.
20	Ochre with white ends	Unramified, straight, 0.5 x 0.4 mm	Covered with soil particles	Concentrated proximally	n.o.
21	Brown	Pyramidal-pinnate, straight, 5 x 0.5 mm	Glistening,	Spiny, not specifically distributed	n.o.
22	Orange with ends	Unramified, straight, 2 x 0.3 mm	Smooth	n.o.	n.o.
23	Brown with white ends	Unramified, straight, 1.0-1.2 mm	Smooth	n.o.	n.o.
24	White	Pyramidal- pinnate, straight, 1 x 0.3 mm	Smooth, milky	n.o.	n.o.
25	Orange with white ends	Monopodial pinnate, slightly bent, 3-4 x 0.5 mm	Smooth	n.o.	n.o.
26	Dark brown	Irregularly -pinnate, straight, 1.5 x 0.2 mm	Densely cottony	Golden, not specifically distributed	n.o.
27	Orange with dark brown spots	2-0.5 mm	Rough	Not specifically distributed	n.o.
28	Fair yellow	Unramified, straight, 1.5-5 x 0.5 mm	Spiny	Not specifically distributed	n.o.
29	Pink	Unramified or dichotomous, 2.5 x 0.3 mm	Loosely cottony	Lacking only at the tip	n.o.
30	Brown with white ends	Monopodial-pinnate, straight 1 x 0.4 mm	Loosely short spiny	Not specifically distributed	n.o.
31	Dark brown	Irregularly-pinnate, straight, 1.5 x 0.5 mm	Spiny	Lacking only at the tip	n.o.
32	Brown with dark spots	Unramified, straight, 0.2 x 0.1 mm	Shiny	n.o.	n.o.
33	Brown	Unramified, sinuous, 1.0 x 0.25 mm	Rough	Not specifically distributed	n.o.
34	Whitish-pink	Dichotomous, bent, 1.0 x 0.25 mm	Transparent, milky	Not specifically distributed	White, round infrequently
35	Dark brown	Unramified, round, 0.4 x 0.25 mm	Loosely woolly	Not specifically distributed	Grey, branched, frequently
36	Dark	Monopodial-pinnate, straight, 0.8-5 x 0.3 mm	Glistening, woolly	Not specifically distributed	Dark, round, frequently
37	Dark pink	Unramified straight, 1 x 0.2 mm	Densely cottony, silvery	Not specifically distributed	n.o.
38	White	Unramified, sinuous, 1.2 x 0.3 mm	Densely cottony, silvery	Not specifically distributed	n.o.

39	Dark orange	Dichotomous, sinuous, 2.5 x 0.4 mm	Shiny, Smooth	n.o.	n.o.
40	White with dark ends	Unramified, bent, 1.5 x 0.4 mm	Long spiny	Not specifically distributed	n.o.
41	Fair yellow	Unramified, pointed, 1.0 x 0.2 mm	Glistening,	n.o.	n.o.
42	Ochre	Unramified, straight, 1.0 x 0.4 mm	Covered with soil particles, glistening	Concentrated proximally	n.o.
43	Reddish	Monopodial-pinnate, straight, 1.5 x 0.25 mm	Striped, shiny, short spiny	Not specifically distributed	n.o.
44	Pale yellow-ochre	Unramified, straight, 1.5 x 0.3 mm	Loosely woolly	Lacking only at the tip	n.o.
45	Dark yellow	Monopodial-pinnate, straight, 1-5 x 0.8 mm	Smooth	n.o.	n.o.
46	Yellow	Unramified, straight, 1.0 x 0.4 mm	Glistening	n.o.	n.o.
47	Whitish	Coralloid, 1 x 0.5 mm	Loosely cottony, glistening	Not specifically distributed	n.o.
48	Yellow with white ends	Unramified or Dichotomous, straight, 0.8 x 0.25 mm	Rough	n.o.	n.o.
49	Ocher	Irregularly-pinnate, bent, 2-3 x 0.5 mm	Loosely cottony	Not specifically distributed	n.o.
50	Grey	Unramified, straight, 1.2 x 0.2 mm	Smooth with stripes	n.o.	n.o.
51	Yellow, red dotted	Unramified, straight, 1 x 0.4 mm	Loosely short spiny	Not specifically distributed	n.o.
52	Dark with white ends	Unramified, straight, 0.8 x 0.2 mm	Densely long spiny	Only at the tip	n.o.
53	Reddish	Unramified, straight, 0.6 x 0.2 mm	Transparent, glistening	Not specifically distributed	White, round, frequently
54	Bluish	Unramified, straight	Loosely cottony	Not specifically distributed	n.o.

Notes: n.o., not observed

APPENDIX C

Characteristics of *F. sylvatica* saplings growing in the three different plots (invaded by *Impatiens glandulifera*, *I. glandulifera* removed, uninvaded) equally distributed over little, moderately and highly disturbed forest areas. Saplings were harvested after 3, 6 and 15 months.

Harvesting time	Intensity of former forest disturbance								
	little			moderate			high		
	invaded	removed	uninvaded	invaded	removed	uninvaded	invaded	removed	uninvaded
After 3 months									
Total biomass (g)	4.53 ± 0.36	5.46 ± 0.38	4.09 ± 0.39	4.34 ± 0.33	5.60 ± 0.63	5.48 ± 0.50	5.42 ± 0.46	8.88 ± 0.6	6.28 ± 0.59
Shoot (g)	2.21 ± 0.20	2.62 ± 0.21	1.91 ± 0.23	2.10 ± 0.18	2.87 ± 0.31	2.54 ± 0.22	2.44 ± 0.23	4.22 ± 0.32	2.97 ± 0.37
Root (g)	2.33 ± 0.18	2.84 ± 0.20	2.17 ± 0.18	2.23 ± 0.19	2.73 ± 0.33	2.94 ± 0.28	2.98 ± 0.27	4.66 ± 0.34	3.32 ± 0.24
Main root (g)	2.15 ± 0.20	2.69 ± 0.20	1.95 ± 0.17	2.09 ± 0.16	2.45 ± 0.30	2.73 ± 0.27	2.77 ± 0.26	4.41 ± 0.33	3.10 ± 0.22
Fine root (mg)	179 ± 27	152 ± 12	220 ± 32	142 ± 25	282 ± 46	205 ± 18	214 ± 44	248 ± 15	218 ± 34
Root/shoot	1.07 ± 0.07	1.10 ± 0.0	1.17 ± 0.08	1.07 ± 0.06	0.95 ± 0.05	1.15 ± 0.04	1.24 ± 0.09	1.11 ± 0.04	1.15 ± 0.07
Fineroot/mainroot	0.09 ± 0.02	0.06 ± 0.01	0.12 ± 0.02	0.07± 0.01	0.12 ± 0.01	0.08 ± 0.00	0.08 ± 0.01	0.06 ± 0.00	0.07 ± 0.01
After 6 months									
Total biomass (g)	4.56 ± 0.45	6.09 ± 1.10	5.90 ± 0.50	4.51 ± 0.44	5.77 ± 0.89	5.29 ± 0.72	7.20 ± 0.85	8.41 ± 1.56	7.81 ± 0.64
Shoot (g)	2.20 ± 0.25	2.77 ± 0.50	2.60 ± 0.21	2.23 ± 0.24	2.81 ± 0.47	2.48 ± 0.37	3.02 ± 0.42	3.13 ± 0.56	3.24 ± 0.25
Root (g)	2.37 ± 0.20	3.32 ± 0.60	3.30 ± 0.35	2.28 ± 0.23	2.95 ± 0.44	2.81 ± 0.37	4.18 ± 0.46	5.27 ± 1.02	4.57 ± 0.40
Main root (g)	2.12 ± 0.19	2.98 ± 0.57	2.90 ± 0.31	2.10 ± 0.23	2.66 ± 0.38	2.51 ± 0.31	3.85 ± 0.43	4.66 ± 0.90	4.14 ± 0.36
Fine root (mg)	242 ± 32	335 ± 52	404 ± 53	175 ± 25	293 ± 75	294 ± 62	332 ± 41	620 ± 126	428 ± 62
Root/shoot	1.10 ± 0.05	1.21 ± 0.07	1.28 ± 0.11	1.04 ± 0.07	1.06 ± 0.07	1.16 ± 0.07	1.45 ± 0.14	1.69 ± 0.15	1.41 ± 0.04
Fineroot/mainroot	0.12 ± 0.02	0.12 ± 0.02	0.14 ± 0.01	0.09 ± 0.02	0.11 ± 0.02	0.12 ± 0.01	0.09 ± 0.01	0.14 ± 0.01	0.10 ± 0.01
After 15 months									
Total biomass (g)	4.63 ± 0.29	8.58 ± 1.22	7.36 ± 0.64	5.31 ± 0.52	8.94 ± 1.08	7.11 ± 0.75	8.09 ± 1.28	10.67 ± 1.65	9.21 ± 1.27
Shoot (g)	2.28 ± 0.18	4.42 ± 0.73	3.36 ± 0.32	2.68 ± 0.34	4.63 ± 0.69	3.49 ± 0.36	3.83 ± 0.51	4.33 ± 0.84	4.33 ± 0.69
Root (g)	2.35 ± 0.11	4.16 ± 0.32	3.99 ± 0.32	2.64 ± 0.18	4.31 ± 0.42	3.62 ± 0.40	4.26 ± 048	5.64 ± 0.86	4.89 ± 0.66
Main root (g)	2.11 ± 0.09	3.40 ± 0.41	3.08 ± 0.22	2.31 ± 0.23	3.45 ± 0.35	2.74 ± 0.28	2.10 ± 0.23	2.66 ± 0.38	2.51 ± 0.31
Fine root (mg)	243 ± 23	765 ± 99	916 ± 135	322 ± 47	863 ± 180	880 ± 135	561 ± 138	1108 ± 269	837 ± 135
Root/shoot	1.05 ± 0.06	1.00 ± 0.09	1.20 ± 0.04	1.04 ± 0.09	0.96 ± 0.06	1.04 ± 0.04	1.16 ± 0.07	1.16 ± 0.09	1.18 ± 0.09
Fineroot/mainroot	0.12 ± 0.01	0.23 ± 0.02	0.30 ± 0.03	0.16 ± 0.04	0.26 ± 0.07	0.32 ± 0.02	0.15 ± 0.03	0.25 ± 0.06	0.21 ± 0.02

Notes: Means ± SE are given, n = 6

CHAPTER 4

Inhibitory potential of naphtoquinones leaching from leaves and exuded from roots of the invasive plant *Impatiens glandulifera*

Regina Ruckli, Katharina Hesse, Gaetan Glauser, Hans-Peter Rusterholz and Bruno Baur

J Chem Ecol (2014) 40:371–378
DOI 10.1007/s10886-014-0421-5

Inhibitory Potential of Naphthoquinones Leached from Leaves and Exuded from Roots of the Invasive Plant *Impatiens glandulifera*

Regina Ruckli · Katharina Hesse · Gaetan Glauser · Hans-Peter Rusterholz · Bruno Baur

Received: 21 November 2013 / Revised: 10 March 2014 / Accepted: 20 March 2014 / Published online: 11 April 2014

Abstract Exploring the effects of allelopathic plant chemicals on the growth of native vegetation is essential to understand their ecological roles and importance in exotic plant invasion. Naphthoquinones have been identified as potential growth inhibitors produced by *Impatiens glandulifera*, an exotic annual plant that recently invaded temperate forests in Europe. However, naphthoquinone release and inhibitory potential have not been examined. We quantified the naphthoquinone content in cotyledons, leaves, stems, and roots from plants of different ages of both the invasive *I. glandulifera* and native *Impatiens noli-tangere* as well as in soil extracts and rainwater rinsed from leaves of either plant species by using ultra-high pressure liquid chromatography-mass spectrometry (UHPLC-MS). We identified the compound 2-methoxy-1,4-naphthoquinone (2-MNQ) exclusively in plant organs of *I. glandulifera*, in resin bags buried into the soil of patches invaded by *I. glandulifera,* and in rainwater rinsed from its leaves. This indicates that 2-MNQ is released from the roots of *I. glandulifera* and leached from its leaves by rain. Specific bioassays using aqueous shoot and root extracts revealed a strong inhibitory effect on the germination of two native forest herbs and on the mycelium growth of three ectomycorrhiza fungi. These findings suggest that the release of 2-MNQ may contribute to the invasion success of *I. glandulifera* and support the novel weapons hypothesis.

Keywords *Impatiens glandulifera* · Chemical defence · 2-methoxy-1,4-naphthoquinone · Ectomycorrhiza · Invasion

Introduction

Invasions of non-native plant species are a major threat for the native biodiversity in many ecosystems (Pejchar and Mooney 2009; see Weidenhamer and Callaway 2010, for a review). Various hypotheses have been proposed to explain the invasiveness of non-native plants (e.g., Bakker and Wilson 2001; Blossey and Notzold 1995; Davis et al. 2000; Keane and Crawley 2002). The novel weapons hypothesis assumes that some invasive plant species produce secondary metabolites that are novel in their non-native ranges, and that this novelty provides advantages to the invasive as it interacts with native plants, microbes, or generalist herbivores (Callaway and Ridenour 2004; Inderjit et al. 2011). An increasing number of studies provide support for the novel weapons hypothesis (Abhilasha et al. 2008; Barto et al. 2010; Callaway et al. 2004; Cantor et al. 2011; Scharfy et al. 2011).

Impatiens glandulifera, an annual plant belonging to the family *Balsaminaceae*, was first introduced from the western Himalaya to Europe as an ornamental garden plant in 1839 (Beerling and Perrins 1993). It became naturalized and invaded riparian habitats in most European countries (Hejda and Pysek 2006). In the last decade, *I. glandulifera* has increasingly invaded deciduous and coniferous forests disturbed by windthrows and/or intensive forest management (Nobis 2008). *Impatiens glandulifera* negatively affects ecosystems by competing successfully with native plants for pollinators, which may reduce native plant fitness (Chittka and Schurkens 2001). *I. glandulifera* also causes changes in the species

Electronic supplementary material The online version of this article (doi:10.1007/s10886-014-0421-5) contains supplementary material, which is available to authorized users.

R. Ruckli · K. Hesse · H.-P. Rusterholz (✉) · B. Baur
Section of Conservation Biology, Department of Environmental Sciences, University of Basel, St. Johanns-Vorstadt 10, CH-4056 Basel, Switzerland
e-mail: hans-peter.rusterholz@unibas.ch

G. Glauser
Chemical Analytical Service of the Swiss Plant Science Web, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

composition of native plant communities in riparian habitats (Hejda and Pysek 2006; Maule et al. 2000). In mixed deciduous forests, *I. glandulifera* influences the litter-dwelling gastropod community by increasing soil moisture and dampening daily soil temperature fluctuations (Ruckli et al. 2013), and suppresses arbuscular mycorrhiza symbiosis in *Acer pseudoplatanus* saplings (Ruckli et al. 2014).

Impatiens glandulifera contains secondary metabolites including flavones, caffeic acid derivatives, and naphthoquinones (oxygen-derivatives of naphthalene) (Lobstein et al. 2001; Šerá et al. 2005). Naphthoquinones have been the subject of medical and ecological studies because of their anticarcinogenic properties (Babula et al. 2009; Devi et al. 1999; Vennerstrom and Eaton 1988) and their inhibitory effects on fungal spore germination (Foote et al. 1949; Yang et al. 2001), bacteria growth (Athip and Pharkphoom 2012), seedling germination and growth (Terzi 2008), and their negative effects on larval development of insects (Lee and Lee 2008; Mitchell et al. 2007). High levels of 2-methoxy-1,4 naphthoquinone (2-MNQ) were detected in leaves of *I. glandulifera*, but not in leaves of the native *Impatiens noli-tangere* (Chapelle 1974; Mitchell et al. 2007). Furthermore, Lobstein et al. (2001) found 2-MNQ and 2-hydroxy-1,4 naphthoquinone (lawsone) in leaves and stems of *I. glandulifera* plants of different ages. However, it is not known whether these naphthoquinones also occur in the roots of *I. glandulifera*.

In general, phytotoxic substances are released from plants to the environment by: (1) exudation from roots, (2) leaching from plants by rain, or (3) decomposition of residues. Aqueous shoot extracts of flowering *I. glandulifera* inhibit germination success and root length of the native plant *Sinapis alba* (Csiszàr et al. 2012), *Leucinapis alba,* and *Brassica napis* (Vrchotová et al. 2011). However, the natural pathway for release of allelopathic substances by *I. glandulifera* and the entire spectrum of inhibitory potential have not yet been examined. In this study, we investigated the way(s) allelopathic naphthoquinones are released from *I. glandulifera* plants, and we explored the inhibitory potential of these substances on the growth of ectomycorrhiza fungi (EM) and the germination of native forest plants by using resin bags, plant extracts, leachate analysis, and bioassays on native plants and fungi. We addressed the following questions: (1) Do different plant organs of the invasive *I. glandulifera* and the native *I. noli-tangere* differ in type and content of naphthoquinones? (2) Do plants of different age (juvenile, flowering, senescent) in each species differ in naphthoquinone content? (3) Do naphthoquinones exude from roots into the soil and/or leach from the foliage by rainwater? (4) Do aqueous shoot and root extracts obtained from juvenile, flowering, and senescent *I. glandulifera* plants contain different amounts of naphthoquinones? (5) Do aqueous shoot and root extracts of the invasive *I. glandulifera* reduce the germination success of native herbaceous plant species and mycelium growth of EM fungi? (6) Does synthetic naphthoquinone affect mycelium growth of EM fungi?

Methods and Materials

Plant Sampling Invasive *I. glandulifera* and native *I. noli-tangere* were sampled in April (seedlings), May and June (juvenile plants), July (flowering plants), September (fruiting plants), October (senescent plants), and in November 2011 (partly decomposed plants) (online resource 1). Samples consisting of 3–7 individuals were collected in an area measuring 50×50 m in a semi-natural beech forest near Basel (47°26′ N, 7°33′ E). The plants were transported on ice to the laboratory, where cotyledons, leaves, stems, and roots were separated and lyophilized for 72 hr (VirTis BenchTop 2K, SP Industries; US). Dried samples were ground with a ball mill (Retsch MM200, Schieritz & Hauenstein AG, Switzerland), and 5–60 mg of the ground material were mixed with 1 ml methanol:water (4:1, v/v) and incubated at ambient temperature for 30 min before centrifuging for 15 min at 16,000 rpm and removing the supernatants for storage at −20 °C.

Sampling of Plant Exudates We used the slightly modified protocol of Ens et al. (2010) to measure the naphthoquinones released by *I. glandulifera* into the soil. Cotton fabric bags (7.5×5 cm) were filled with 10 g of Amberlite® XAD4 (Sigma-Aldrich, Switzerland), washed with distilled water, and then twice with dichloromethane (DCM, HPLC grade), dried for 24 hr at 40 °C, and stored in an air-tight glass jar prior to exposure in soil.

To assess the effectiveness of the resin bag technique, we measured the percentages of adsorption and recovery of 2-MNQ released in water by placing five resin bags in a 0.01 % 2-MNQ (Sigma-Aldrich, Switzerland) solution for 24 hr. Adsorption averaged 74.0±5.6 %, and subsequent recovery with DCM after 24 hr was 90.1±6.3 %. This indicates that the effectiveness of the resin bag technique was approximately 67 %.

Exudates released by *I. glandulifera* plants of different ages were sampled in three areas adjacent to the sampling site described above. In each forest area, three homogeneous patches invaded by *I. glandulifera* and three patches not colonized by the plant were selected. In each patch, two resin bags were buried 10–15 cm below ground surface. Bags were exposed for 20 days from 17 May to 6 June 2011 (seedling and juvenile stage), 17 July to 7 August 2011 (flowering stage), 18 October to 7 November 2011 (senescent stage), and 16 January to 6 February 2012 (partly decomposing stage; online resource 1). Afterwards, the bags were transported on ice to the lab and stored at −80 °C. Some bags were lost: in all 121 out of 144 bags were recovered.

To extract the adsorbed plant exudates, we placed 5 bags into a conical flask containing 250 ml DCM. The sealed flasks were shaken at ambient temperature for 24 hr, the solution was filtered and evaporated to dryness under reduced pressure at 40 °C. The residue was mixed with 1 ml methanol:water (4:1, v/v),incubated at ambient temperature for 30 min, and centrifuged for 15 min at 16,000 rpm. Before recovery, the supernatants were stored at −20 °C until required. The controls were five clean resin bags that had been kept in a sealed glass jar for 20 days.

Rainwater Sampling To examine whether naphthoquinones of *I. glandulifera* could be leached by rain, we collected rainwater during the flowering stage of the plant on 2 days in July 2011 (online resource 1). Five patches with *I. glandulifera* and five patches with no invasive plants were chosen. A plastic tray (21×9×10 cm) covered with a net to prevent contamination by falling foliage was placed prior to rainfall both under *I. glandulifera* (in invaded plots) and under native vegetation (in uninvaded plots). Samples of 50 ml of rainwater were obtained from each plastic tray immediately after an abundant rain, transferred to separatory funnels, and mixed with 50 ml DCM in order to collect the organic-soluble phase for analyses as described below.

Mass Spectrometry Analyses Naphthoquinones were analyzed by ultra-high pressure liquid chromatography/mass spectrometry (UHPLC/MS) using an Acquity UPLC™ system (Waters, Milford, MA, USA) coupled to a Synapt G2 MS QTOF (Waters, Milford, MA) equipped with an atmospheric pressure chemical ionization (APCI) source. An Acquity BEH C18 column (50×2.1 mm, 1.7 μm) was used under the following conditions: solvent A = water; solvent B = acetonitrile; 5–52.5 % B in 2.0 min, 52.5–100 % B in 0.5 min, holding at 100 % B for 1.0 min, re-equilibration at 5 % B for 0.9 min. The flow rate was set to 700 μl min^{-1}, and the injection volume was 2.5 μl. The temperatures of the column and of the autosampler chamber were maintained at 30 and 15 °C, respectively. Data were acquired with a scan time of 0.4 sec over an *m/z* range of 85–600 Da in the negative ion MS mode. The corona current was set to 20 μA and the cone voltage to 30 V. The source temperature was maintained at 120 °C and the APCI probe temperature at 370 °C. The desolvation gas flow was set to 800 L hr^{-1}. To quantify the different naphthoquinones in each sample, standard calibration curves obtained from different concentrations of synthetic 1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, 5-hydroxy-1,4-naphthoquinone, and 2-methoxy-1,4-naphthoquinone (Sigma-Aldrich, Switzerland) were used.

Preparation of Aqueous Extracts for Bioassays Shoot and root material of *I. glandulifera* was collected from juvenile plants in May, from flowering plants in July and senescent plants in October 2011 (online resource 1). The tissue samples (300 g fresh weight) were soaked in 1 l of distilled water at ambient temperature for 48 hr, filtered twice through cheesecloth and then under pressure through a Millipore®-membrane (0.45 μm) to prevent potential contamination by microorganisms. Three samples of 1 ml of each stock solution were stored at −20 °C.

Seed Bioassays The phytotoxic effect of aqueous shoot and root extracts of *I. glandulifera* on the germination of two native plants (*Hieracium murorum* and *Scrophularia nodosa*) growing in the same forest was tested in a seed bioassay. Stock extracts (shoot or root) of juvenile, flowering and senescent *I. glandulifera* were diluted with distilled water to obtain concentrations of 0.250, 0.125, and 0.0625 g of fresh weight of plant tissue per ml.

Twenty-five surface-sterilized seeds of *H. murorum* or *S. nodosa* were placed on a filter paper in a petri dish (90 mm, Roth AG, Switzerland) before adding 3 ml of test solutions or distilled water (control) and sealing the dishes twice with parafilm for incubation in a climate chamber at 22/15 °C with a light:dark cycle of 16:8 hr for 20 days. All dishes were checked for germinated seeds at the end of the experiment. Three replicates of each test solution and control were prepared for each plant species, resulting in a total of 126 petri dishes. Inhibition/stimulation on germination was calculated following Chung et al. (2001):

$$\text{inhibition/stimulation} = (t-c/c) \times 100(\text{in } \%)$$

where t is the percentage germination measured in dishes exposed to different extract concentrations and c represents the mean of the percentage germination of the corresponding controls.

Mycelium Growth Bioassays Potential inhibitory effects of aqueous shoot and root extracts of juvenile, flowering, and senescent *I. glandulifera* were tested on the mycelium growth of three EM fungi (*Pisolithus tinctorius, Lactarius subdulci,* and *Laccaria bicolore*) typical for beech forests. Modified Melin-Norkrans (MMN) agar (Marx 1969) containing the same concentrations of plant tissue extracts (0.250, 0.125, and 0.0625 g fresh weight per ml agar) as used in the seed bioassay was prepared, and used to fill Petri dishes with 20 ml agar. As a control, MNN agar was prepared with sterilized water. A fungal plug (diam 4 mm) was placed in the center of each petri dish before sealing the dishes twice with parafilm. Three replicates of each agar solution and control were prepared for each fungal species, resulting in a total of 189 petri dishes.

Table 1 Amount (mean ± SE) of 2-methoxy-1,4-naphthoquinone (2-MNQ) and 1,4-naphthoquinone (NQ) determined in resin bags buried for 20 days in the soil 5–10 cm from *Impatiens glandulifera* plants of different ages

Plant age	2-MNQ [μg per bag]	NQ [μg per bag]	Number of bags
Seedling/juvenile	0.701±0.273	0.483±0.289	31
Flowering	0.321±0.167	0.295±0.098	36
Senescent	1.304±0.365	0.216±0.067	36
Decomposed	0.102±0.102	not detected	18

Dishes were incubated in a climate chamber at 26 °C in darkness for 45 days. The area covered by the mycelium (mm^2) was recorded by drawing its circumference on the top of each petri dish at the end of the experiment, and its area was measured by using Image J (Rasband 1997–2012). Inhibition/stimulation of mycelium growth was calculated as described above.

Synthetic 2-Methoxy-1,4-Naphthoquinone on Mycelium Growth MMN agar solutions containing 0.1, 0.3, 1, and 3 μg of synthetic 2-MNQ (Sigma-Aldrich, Switzerland) per ml agar were prepared to assess its phytotoxic effect on the mycelium growth of the three EM fungi at concentrations similar to the levels in soils, based on the resin bag analysis (Table 1) and the levels in aqueous shoot extracts of the invasive plant (Table 2). For each EM fungi, we prepared three replicates of each concentration and control resulting in a total of 45 dishes. Inhibition was calculated as in the mycelium growth bioassay.

Statistical Analyses All analyses were carried out using R (R Development Core Team 2012, version 2.15.1). ANOVA was used to examine the effects of *I. glandulifera* plant stage (juvenile, flowering, and senescent), type of plant material (root or shoot), and concentration of extracts (0.0625, 0.125, and 0.250 g plant material/ml) on inhibition/stimulation (in %) of germination of *H. murorum* and *S. nodosa* or on mycelium growth of *L. bicolore*, *L. subdulci,* and *P. tinctorius*. Student's *t*-tests were used to examine whether the inhibition/stimulation (in %) of the different extract concentrations significantly deviated from zero. Spearman rank correlation was applied to examine whether inhibition/stimulation (%) was related to the 2-MNQ content found in aqueous shoot extracts of all plant stages of *I. glandulifera*.

Table 2 Amount (mean ± SE, *N*=5) of 2-methoxy-1,4-naphthoquinone (2-MNQ) determined in aqueous shoot extracts of *Impatiens glandulifera* of different ages (300 g fresh weight of shoot tissue extracted with 1 l of water)

Plant age	2-MNQ [μg per ml]
Seedling/juvenile	14.64±1.55
Flowering	9.03±0.07
Senescent	4.41±0.14

Results

Naphthoquinones in Plants 2-Methoxy-1,4-naphthoquinone (2-MNQ) was identified in leaves, stems, and roots of *I. glandulifera*, but not in cotyledons (Fig. 1a–c). Overall, the concentration of 2-MNQ (% dry weight) in leaves of *I. glandulifera* was up to 10–15 times higher than in roots and stems and declined with plant age (Fig. 1a–c). In contrast, 2-hydroxy-1,4-naphthoquinone (lawsone) was found only in roots of *I. glandulifera* in the senescent stage. In the native

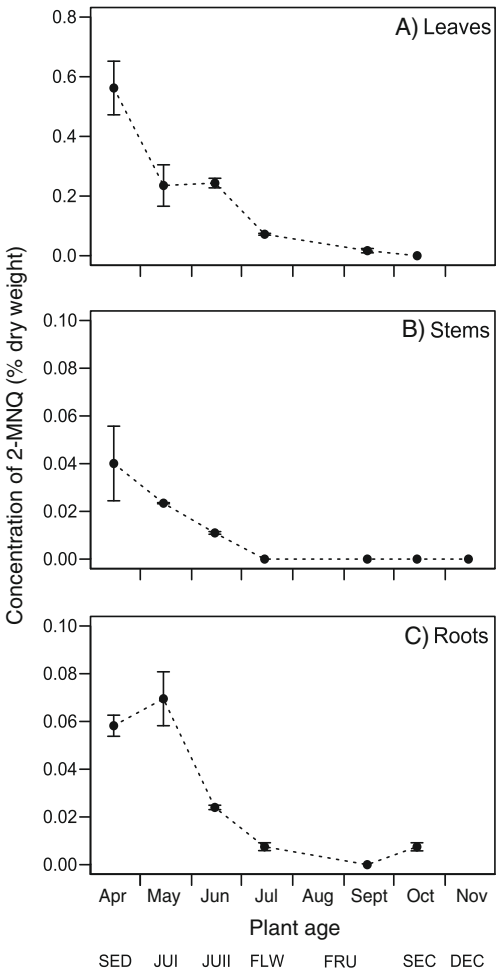

Fig. 1 Concentrations of 2-methoxy-1,4-naphthoquinone (2-MNQ) in leaves (**a**), stems (**b**), and roots (**c**) of *Impatiens glandulifera*, for plants of different ages. *SED* seedlings in April; *JUI* juveniles in May; *JUII* juveniles in June; *FLW* flowering plants in July; *FRU* fruiting plants in August; *SEC* senescent plants in October; *DEC* decomposing plants in November

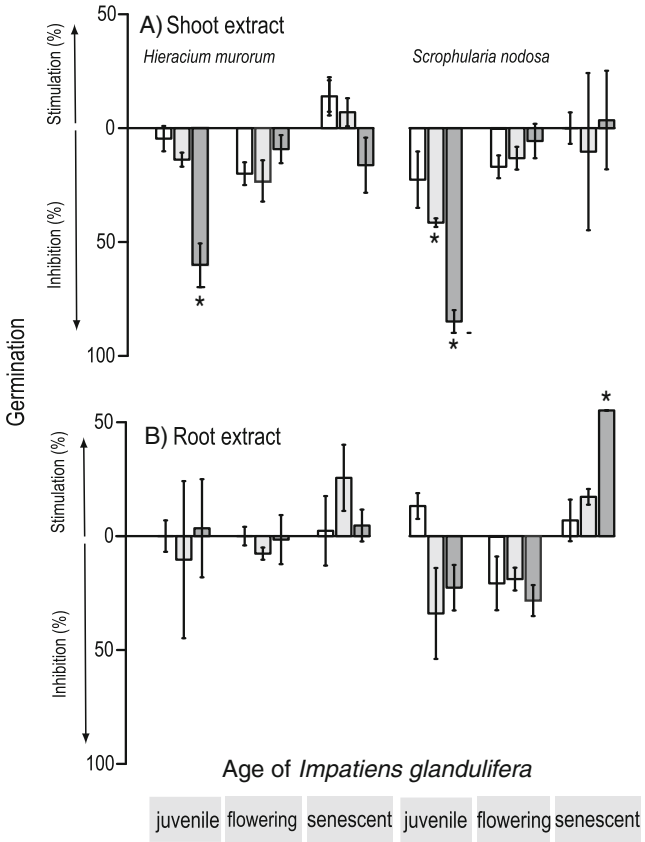

Fig. 2 Inhibition/stimulation of seed germination (%) (mean ± SE) for *Hieracium murorum* and *Scrophularia nodosa* by aqueous shoot (**a**) or root (**b**) extracts of *Impatiens glandulifera* plants of different ages. Concentrations of shoot or root tissue in different extracts were 0.0625 g/ml (*white bars*), 0.125 g/ml (*light grey bars*), and 0.250 g/ml (*dark grey bars*). Values are presented as percentage difference to the controls. Student's *t*-test was used to examine significant deviations from zero (* indicates $P<0.05$)

I. noli-tangere, lawsone was identified in leaves of flowering (mean ± SE in each case, $N=5$; 0.018±0.005 %) and fruiting (0.082±0.005 %) individuals and in roots of senescent plants (0.012±0.002 %).

Naphthoquinones in Soil Extracts, Rainwater, and Aqueous Extracts 2-MNQ and 1,4-naphthoquinone (NQ) from plant exudates of *I. glandulifera* were determined by using resin bags (Table 1). The amount of 2-MNQ determined per bag varied among plants of different ages. In contrast, the amount of NQ declined with plant age (Table 1). 2-MNQ and NQ were not detected in resin bags buried under native forest vegetation.

2-MNQ also was found in rainwater rinsed from *I. glandulifera* leaves (mean ± SE = 12.21± 3.01 μg ml^{-1}), but not in rainwater rinsed from native forest vegetation.

In aqueous extracts of *I. glandulifera* shoots, the concentration of 2-MNQ (μg/ml) declined from the juvenile to the senescent stage (Table 2). However, 2-MNQ was not detected in the aqueous root extracts.

Seed Bioassays Germination of *H. murorum* and *S. nodosa* seeds was inhibited to a different extent by aqueous extracts obtained from *I. glandulifera* plants of different ages (Fig. 2, Tukey HSD tests $P<0.05$, online resource 2). Shoot extracts of the invasive plant had a significantly higher inhibitory effect on seed germination of both native species than root extracts (Fig. 2, online resource 2). Only the most concentrated shoot extract obtained from juvenile plants significantly inhibited seed germination of *H. murorum* (Fig. 2). Similarly, seeds of *S. nodosa* were significantly inhibited by 42 and 85 % in the two most concentrated aqueous shoot extracts obtained from juvenile plants (Fig. 2). In contrast, the germination of *S. nodosa* seeds was differently affected by root extracts obtained from plants of different ages. The most concentrated root extract obtained from senescent plants resulted in a 55 % stimulation of seed germination in *S. nodosa* (Fig. 2, online resource 2). Inhibition of seed germination (%) in both species increased with increasing 2-MNQ concentrations in shoot extracts (*H. murorum*; $r_s=-0.633$, $N=18$, $P<0.001$; *S. nodosa;* $r_s=-0.503$, $N=18$, $P<0.001$; online resource 3).

Mycelium Growth Bioassays Aqueous extracts inhibited mycelium growth (%) in all three EM species (*L. bicolore*, *L. subdulci* and *P. tinctorius*) with a significant dependence on the age of the invasive plant (Tukey HSD tests $P<0.05$, online resource 4). Shoot extracts had a significantly higher inhibitory effect on mycelium growth than root extracts (Fig. 3, online resource 4). All concentrations of shoot extracts from juvenile plants inhibited mycelium growth by 100 %, while corresponding root extracts did not affect mycelium growth of the fungi examined (Fig. 3, online resource 4). Moreover, mycelium growth of *L. bicolore* and *P. tinctorius* was significantly inhibited by root extracts from flowering and senescent plants (Fig. 3), independent of their concentrations. In contrast, inhibition of mycelium growth (%) in *L. subdulci* was significantly reduced by the most concentrated root extract from flowering plants and as well as by root extracts from senescent plants (Fig. 3).

Mycelium growth decreased with increasing 2-MNQ concentrations in aqueous shoot extracts (*L. bicolore*; $r_s=-0.826$, $N=18$, $P<0.001$; *L. subdulci*; $r_s=-0.837$, $N=18$, $P<0.001$; *P. tinctorius*; $r_s=-0.845$, $N=18$, $P<0.001$; online resource 5).

Synthetic 2-MNQ Mycelium growth of all three fungal species was significantly inhibited when synthetic 2-MNQ was added to the growth medium. In all species, fungal growth decreased with increasing 2-MNQ concentration (Fig. 4, online resources 4).

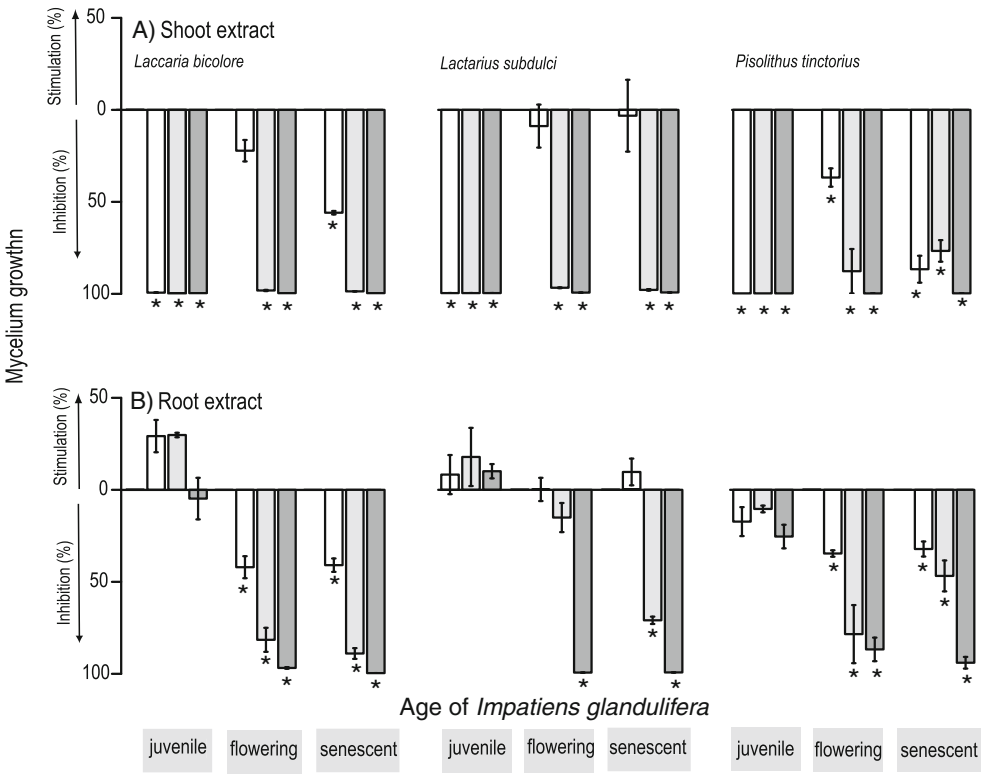

Fig. 3 Inhibition/stimulation of mycelium growth (%) (mean ± SE) for the fungi *Laccaria bicolore*, *Lactarius subdulci,* and *Pisolithus tinctorius* by aqueous shoot (**a**) or root (**b**) extracts obtained from *Impatiens glandulifera* plants of different ages. Concentrations of shoot or root tissue in extracts were 0.0625 g/ml (*white bars*), 0.125 g/ml (*light grey bars*) and 0.250 g/ml (*dark grey bars*). Values are presented as percentage difference to the controls. Student's *t*-test was used to examine significant deviations from zero (* indicates $P<0.05$)

Discussion

We observed organ-specific occurrence of 2-MNQ with the highest concentrations in leaves, confirming previous studies (Chapelle 1974; Lobstein et al. 2001) Furthermore, our study demonstrated 2-MNQ in roots of *I. glandulifera* for the first time. The 2-MNQ concentrations found in leaves of the invasive plant were 21 times higher than those detected in leaves of the native *I. noli-tangere* (Lobstein et al. 2001). The high 2-MNQ concentration in leaves could protect the invasive plant against phytophageous insects (Mitchell et al. 2007).

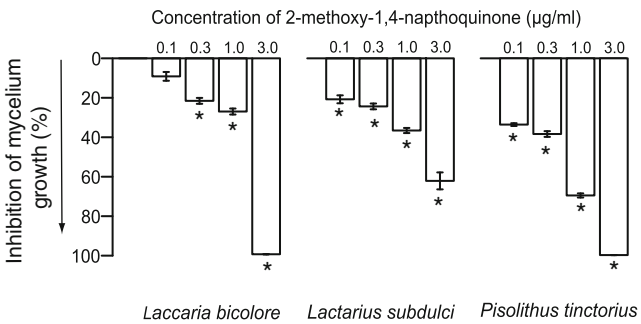

Fig. 4 Inhibition of mycelium growth (%) (mean ± SE) for the fungi *Laccaria bicolore*, *Lactarius subdulci,* and *Pisolithus tinctorius* in response to different 2-MNQ concentrations. Values are presented as percentage difference to the controls. Student's *t*-test was used to examine significant deviations from zero (* indicates $P<0.05$)

The 2-MNQ content of leaves, stems, and roots decreases with plant age, and is highest in seedlings. Thus, 2-MNQ may facilitate the establishment of young invasive plants because the germination of *I. glandulifera* occurs later in the season than the germination of most native plants in this habitat.

In the present study, lawsone was detected only in low quantities in root tissue of *I. glandulifera* in the senescent stage. Similarly, Chapelle (1974) found no lawsone in *I. glandulifera* leaves. In contrast, Lobstein et al. (2001) detected high quantities of lawsone in leaves, stems, and flowers of *I. glandulifera.* In the native species *I. noli-tangere*, lawsone was not detected in leaves in our study or by Chapelle (1974). However, our results contrast with those of Lobstein et al. (2001) who found low quantities of this naphthoquinone in flowering plants. Most probably, the differences in quantities and types of naphthoquinone reported are a result of different sample preparation methods and analytical techniques used for the determination of naphthoquinone content.

Our results show that 2-MNQ is released from the invasive plant by exuding from roots and leaching from leaves. Rainwater rinsed from *I. glandulifera* leaves contained 2-MNQ, while water rinsed from native vegetation did not contain this compound. Foliage leaching of allelopathic compounds has been demonstrated in other plants, e.g., juglone from *Juglans nigra* (Rietveld 1983) and canopy leachates from *Acacia dealbata*, an invasive tree (Lorenzo et al. 2011). Furthermore, several invasive plants exude allelopathic compounds from

roots (e.g. benzyl-isothiocyanate, Vaughn and Berhow 1999; (±)catechin, Callaway and Aschehoug 2000; 8-hydroxyquinoline, Inderjit et al. 2010). However, the present study is the first to demonstrate *in situ* the release of a potential allelopathic compound from both leaves and roots. Our data suggest that *I. glandulifera* modifies the chemical composition of the soil.

We found NQ in resin bags buried under invasive plants but could not detect it in any plant organ of *I. glandulifera*. Soil microbes modify chemical compounds that are released from plants into the soil (Inderjit 2005). Thus, soil microbes could degrade 2-MNQ to NQ by an unknown pathway. Degradation of the naphthoquinone juglone has been described (Müller and Lingens 1988; Rettenmaier and Lingens 1985). The seasonal variation of naphthoquinone amounts recorded in plant exudates of invaded soils could be explained partly by the seasonal variation in the activity of the microbial community (Kauri 1982).

Aqueous extracts frequently are used to examine the inhibitory effect of a compound on other organisms (e.g., Dorning and Cipollini 2006; Sun et al. 2006). It is essential to test different concentrations of the compound to entirely explore its inhibitory potential. In our study, the concentration of 2-MNQ found in aqueous extracts of *I. glandulifera* shoots declined in the course of the growing season. Surprisingly, we did not detect 2-MNQ in aqueous root extracts of plants of any age, although we found it in methanolic extracts of root tissues, suggesting that 2-MNQ stored in root tissues is less efficiently extracted in water than in methanol (80 %).

It previously has been shown that aqueous shoot extracts obtained from flowering *I. glandulifera* inhibit the germination of *Sinapis alba* (Csiszàr et al. 2012), *Leucinapis alba,* and *Brassica napis* (Vrchotová et al. 2011). In our study, high concentrations of shoot extracts from juvenile *I. glandulifera* had an inhibitory effect on the germination of the native forest plants *H. murorum* and *S. nodosa*. In contrast, aqueous extracts obtained from flowering *I. glandulifera* shoots and roots did not affect germination of either plant. The results correlate well with the concentrations of 2-NMQ measured in extracts used for the bioassays. Furthermore, *H. murorum* and *S. nodosa* are generalist species and may, therefore, react less sensitively to phytotoxic compounds than more specialized plant species. Our study extends the present knowledge on the inhibitory effect of 2-MNQ by showing that high concentrations of this compound inhibit germination of native forest plants.

Ectomycorrhiza fungi are sensitive to allelochemical compounds in leaf litter, plant tissue and roots (Cote and Thibault 1988; Rice 1979; Rose et al. 1983). In the invasive plant *Alliaria petiolata,* allelopathic compounds disrupt the association between EM fungi and their hosts (Wolfe et al. 2008). 2-MNQ is known for its allelopathic effect on fungal spore germination (Foote et al. 1949; Yang et al. 2001). In our study, EM fungi responded more sensitively than seeds of the plants species examined when exposed to the same concentrations of shoot and root extracts of *I. glandulifera*. Furthermore, mycelium growth of all fungi was affected to a similar extent by extracts of *I. glandulifera*. In general, shoot extracts had a stronger inhibitory effect on mycelium growth than root extracts. Extracts from roots of juvenile *I. glandulifera* were not inhibitory to mycelium growth of the EM species examined. This suggests that compounds other than 2-MNQ are active inhibitors of mycelium growth during the flowering and senescent stages of *I. glandulifera*. For example, degraded phenolic compounds could contribute to the reduction of mycelium growth.

Invasive plants including *Alliaria petiolata* and *Centaurea maculosa* produce allelopathic compounds that are novel in their non-native range and reduce the germination and growth of native plants (Callaway and Ridenour 2004; He et al. 2009). This phenomenon has been described by the novel weapons hypothesis. A similar mechanism could be responsible for the invasion success of *I. glandulifera*. The negative effect of 2-MNQ on herb germination and mycelium growth, and the absence of the compound in soils without *I. glandulifera* suggest that 2-MNQ could indeed be a "novel chemical weapon".

Acknowledgments We thank Anette Baur, Günter Hoch, Evelyn Meyer, and two anonymous reviewers for comments on the manuscript. Financial support was received from the Velux Foundation, Zürich.

References

Abhilasha D, Quintana N, Vivanco JM, Joshi J (2008) Do allelopathic compounds in invasive *Solidago canadensis* restrain the native European Flora? J Ecol 96:993–1001

Athip S, Pharkphoom P (2012) Comparison of antimicrobial activities of naphthoquinones from *Impatiens balsamina*. Nat Prod Res 12: 1119–1124

Babula P, Vojtech A, Kizek R, Sladky Z, Havel L (2009) Naphthoquinones as allelochemical triggers of programmed cell death. Environ Exp Bot 65:330–337

Bakker J, Wilson S (2001) Competitive abilities of introduced and native grasses. Plant Ecol 157:117–125

Barto EK, Powell JR, Cipollini D (2010) How novel are the chemical weapons of garlic mustard in North American forest understories? Biol Invasions 12:3465–3471

Beerling DJ, Perrins JM (1993) *Impatiens glandulifera* Royle (*Impatiens roylei* Walp.). J Ecol 81:367–382

Blossey B, Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants—a hypothesis. J Ecol 83:887–889

Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Science 290: 521–523

Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. Front Ecol Environ 8:436–443

Callaway RM, Thelen GC, Barth S, Ramsey PW, Gannon JE (2004) Soil fungi alter interactions between the invader *Centaurea maculosa* and North American natives. Ecology 85:1062–1071

Cantor A, Hale A, Aaron J, Traw MB, Kalisz S (2011) Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. Biol Invasions 13:3015–3025

Chapelle JP (1974) 2-methoxy-1,4-naphthoquinone in *Impatiens glandulifera* and related species. Phytochemistry 13:662

Chittka L, Schurkens S (2001) Successful invasion of a floral market–an exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature 411:653–653

Chung IM, Ahn JK, Yun SJ (2001) Assessment of allelopathic potential of barnyard grass *(Echinochloa crus-galli*) on rice (*Oryza sativa* L.) cultivars. Crop Prot 20:921–928

Cote JF, Thibault JR (1988) Allelopathic potential of raspberry folliar leachates on growth of ectomycorrhizal fungi associated with black spruce. Am J Bot 75:966–970

Csiszàr A, Korda M, Schmidt D, Šporcic D, Teleki B, Tiborcz V, Zagyvai G, Bartha D (2012) Study on allelopathic potential of some invasive and potentially invasive neophytes. International Scientific Conference March 26–27 2012 on Sustainable Development & Ecological Footprint

Davis MA, Grime JP, Thompson K (2000) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Devi PU, Solomon FE, Sharadea AC (1999) Plumbagin, a plant naphthoquinone with antitumor and radiomodifying properties. Pharm Biol 37:231–236

Dorning M, Cipollini D (2006) Leaf and root extracts of the invasive shrub, *Lonicera maackii*, inhibit seed germination of three herbs with no autotoxic effects. Plant Ecol 184:287–296

Ens EJ, French K, Bremner JB, Korh J (2010) Novel technique shows different hydrophobic chemical signatures of exotic and indigenous plant soils with similar effects of extracts on indigenous species seedling growth. Plant Soil 326:403–414

Foote MW, Little JE, Sproston TJ (1949) On naphthoquinones as inhibitors of spore germination of fungi. J Biol Chem 181:481–487

He WM, Feng YL, Ridenour WM, Thelen GC, Pollock JL, Diaconu A, Callaway RM (2009) Novel weapons and invasion: biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (+/−)-catechin. Oecologia 159:803–815

Hejda M, Pysek P (2006) What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? Biol Conserv 132: 143–152

Inderjit (2005) Soil microorganisms: an important determinant of allelopathic activity. Plant Soil 247:227–236

Inderjit, Bajpai D, Rajeswari MS (2010) Interaction of 8-hydroquinoline with soil environment mediates its ecological function. PLoS ONE 5(9):e12852

Inderjit, Wardle AW, Karban R, Callaway RM (2011) The ecosystem and evolutionary context of allelopathy. Trends Ecol Evol 26:655–662

Kauri T (1982) Seasonal fluctuation in numbers of aerobic bacteria and their spores in four horizons of beech forest soils. Soil Biol Biochem 14:185–190

Keane RM, Crawley MJ (2002) Exotic plants invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Lee CH, Lee HS (2008) Acaricidal activity and function of mite indicators using plumbagin and its derivatives isolated from *Diospyros kaki* Thunb. Roots (*Ebenaceae*). J Microbiol Biotechnol 18:314–321

Lobstein A, Brenne X, Feist E, Metz N, Weniger B, Anton R (2001) Quantitative determination of naphthoquinones of *Impatiens* species. Phytochem Anal 12:202–205

Lorenzo P, Palomera-Pérez A, Reigosa MJ, Gonzàlez L (2011) Allelopathic interference of invasive *Acacia deabata* Link on the physiological parameters of native understory species. Plant Ecol 212:403–412

Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infection. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163

Maule H, Andrews M, Watson C, Cherrill AJ (2000) Distribution, biomass and effect on native species of *Impatiens glandulifera* in a deciduous woodland in northeast England. Asp Appl Biol 58:31–38

Mitchell MJ, Brescia AI, Smith SL, Morgan ED (2007) Effects of the compounds 2-methoxynaphthoquinone, 2-propoxynaphthoquinone, and 2-isopropoxy-naphthoquinone on ecdysone 20-monooxygenase activity. Arch Insect Biochem 66:45–52

Müller U, Lingens F (1988) Degradation of 1,4-naphthoquinones by *Pseudomonas putida*. Biol Chem Hoppe Seyler 369:1031–1104

Nobis M (2008) Invasive Neophyten auch im Wald? Wald Holz 8:46–49

Pejchar L, Mooney HA (2009) Invasive species, ecosystem services and human well-being. Trends Ecol Evol 24:497–504

Rasband WS (1997–2012) ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA

Rettenmaier H, Lingens F (1985) Purification and some properties of two isofunctional juglone hydroxylases from *Pseudomonas putida* J1. J Biol Chem 366:637–646

Rice EL (1979) Allelopathy: an update. Bot Rev 45:15–109

Rietveld WJ (1983) Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. J Chem Ecol 9: 295–308

Rose SL, Perry DA, Pilz D, Schoeneberger MM (1983) Allelopathic effects of litter on the growth and colonization of mycorrhizal fungi. J Chem Ecol 9:1153–1162

Ruckli R, Rusterholz HP, Baur B (2013) Invasion of *Impatiens glandulifera* affects terrestrial gastropods by altering microclimate. Acta Oecol 47:16–23

Ruckli R, Rusterholz HP, Baur B (2014) Invasion of an annual exotic plant into deciduous forests suppresses arbuscular mycorrhiza symbiosis and reduces performance of sycamore. Forest Ecol Manag 318:285–293

Scharfy D, Funk A, Venterink HO, Güsewell S (2011) Invasive forbs differ functionally from native graminoids, but are similar to native forbs. New Phytol 189:818–828

Šerá B, Vrchotová N, Tříska J (2005) Phenolic compounds in the leaves of alien and native *Impatiens* plants. In: Alford DV, Backhaus GF (eds) Plant protection and plant health in Europe: introduction and spread of invasive species. British Crop Production Council, Alton, pp 281–282

Sun BY, Tan JZ, Gu FG, Zhu MD (2006) Allelopathic effects of extracts from *Solidago canadensis* L. against seed germination and seedling growth of some plants. J Environ Sci 7:285–292

Terzi I (2008) Allelopathic effects of juglone and decomposed walnut leaf juices on muskmelon and cucumber seed germination and seedling growth. Afr J Biotechnol 7:1870–1874

Vaughn SF, Berhow MA (1999) Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). J Chem Ecol 25:2495–2504

Vennerstrom JL, Eaton JW (1988) Oxidants, oxidant drugs and malaria. J Med Chem 31:1269–1277

Vrchotová N, Šerá B, Krejčová J (2011) Allelopathic activity of extracts from *Impatiens* species. Plant Soil Environ 2:57–60

Weidenhamer JD, Callaway RM (2010) Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. J Chem Ecol 36:59–69

Wolfe BE, Rodgers VL, Stinson KA, Pringle A (2008) The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. J Ecol 96:777–783

Yang X, Summerhurst DK, Kobal SF, Ficker C, Smith ML, Bernards MA (2001) Isolation of an antimicrobial compound from *Impatiens balsamina* L. using bioassay-guided fractionation. Phytother Res 15:676–680

GENERAL DISCUSSION

The presence of *I. glandulifera* affects above- and below ground organisms by direct and indirect mechanisms. Changes in soil conditions affect gastropod richness and abundance directly whereas allelophatic compounds leaching from leaves of the invasive plant and exuding from roots indirectly reduces mycelium growth of ectomycorrhiza fungi.

In my study, soil moisture was higher in invaded plots and in plots in which *I. glandulifera* was removed than in uninvaded plots. Thus, it is not surprising that I found more gastropod species in patches which retained soil moisture. In the highly disturbed area, fluctuations of daily air and soil temperature were dampened in invaded plots compared to the other plot types (**Chapter 1**). Under these conditions, dense stands of *I. glandulifera* may providing a favourable microclimate for terrestrial gastropods with higher soil moisture and lower temperature fluctuations (Boag 1990, Hawkins et al. 1998). Thus, several gastropod species might be attracted by the moister conditions in areas invaded by *I. glandulifera*, resulting in higher gastropod richness and abundance. However, I could also show in **Chapter 1** that gastropod communities in invaded plots were slightly altered in invaded plots and the proportion of typical forest species was reduced in these plots. Thus, the presence of *I. glandulifera* has the potential to alter the gastropod community at the expense of native forest species. This could influence ecological habitat functions, because gastropods play an important role as detritivores of leaf litter and senescent plants (Mason 1970, Wallwork 1976). Like gastropod diversity, mycorrhizal fungi are indicators for changes in habitat conditions. In **Chapter 3**, I demonstrated that ectomycorrhizal (EM) morphotype communities were changed with a reduced diversity of EM morphotypes on *F. sylvatica* saplings fine roots growing in invaded plots. Different EM morphotypes vary in ecological functions, e.g. in the uptake of specific nutrients (Agerer 2001, Erland and Taylor 2002). Thus, a low diversity of EM morphotypes has negative effects on the nutrient uptake, water supply and growth of numerous trees (Baxter and Dighton 2001, 2005). The observed decline in EM morphotypes diversity on *F. sylvatica* saplings in invaded plot might be a result of the reduced EM colonisation on *F. sylvatica* saplings. Saplings with low colonisation might be more vulnerable to pathogen infections and might have less access to nutrients, resulting in a reduced survival rate and biomass of saplings. Similarly, I found a decreased colonisation rate and both reduced survival and biomass in saplings of *A. pseudoplatanus* (**Chapter 2**).

Measured changes in soil conditions did not affect mycorrhizal colonisation and performance of both sapling species. Therefore other mechanisms may contribute to the decrease of mycorrhizal colonisation and the reduction of the saplings' performance. Interestingly, saplings growing in plots from which *I. glandulifera* was regularly removed showed similar mycorrhizal colonisation and survival rates as control plots. This suggests that allopathic effects of *I. glandulifera* could inhibit mycorrhizal symbiosis of saplings. Mycorrhizal fungi were known to react sensitively to allelochemicals compounds detected in leaf litter, plant and roots (Rice 1979, Rose et al. 1983, Cote and Thibault 1988). In **Chapter 4** I found the allelophatic compound 2-methoxy-1,4-naphtoquinone (2-MNQ) in different plant parts of *I. glandulifera*. 2-MNQ is released from the invasive plant by exuding from roots and leaching from leaves. Leave leaching of allelopathic compounds has been demonstrated in other plant species, e.g. Juglone from *Juglans nigra* (Rietveld 1983) and canopy leachates from *Acacia dealbata*, an invasive tree (Lorenzo et al. 2011). Furthermore, several invasive plants exude allelopathic compounds from roots (e.g. benzyl-isothiocynat, Vaughn and Berhow 1999; (±)catechin, Callaway and Aschehoug 2000; 8-hydroxquinoline, Inderjit et al. 2010). Furher, in a laboratory experiment synthetic 2-MNQ affected mycelium growth in a similar negative way as aqueous extracts obtained from *I. glandulifera* containing 2-MNQ. This indicates that the antifungal 2-MNQ plays a curial role in the inhibition of mycelium growth. Considering the field experiments presented in **Chapter 2 and 3**, 2-MNQ exudate by roots and leached from leaves of *I. glandulifera* could lead to the reduced mycorrhizal colonisation on the saplings examined. The additional negative effects of 2-MNQ on herb germination and the absence of the compound in soils without *I. glandulifera* indicates that 2-MNQ is an candidate for a "novel chemical weapon". However, other hypotheses about the success of invasive plant cannot be excluded. For *I. glandulifera*, also disturbance seems to play an important role for its spread.

The spread of *I. glandulifera* in deciduous forests is a serious threat for forest diversity and forest regeneration, especially given that mycorrhizal symbiosis is a key factor that determines the diversity of plant communities, succession dynamics as well as the resource transport within the ecosystem (Smith and Read, 2009). The combined effects of altered soil properties, deceased mycorrhizal colonisation of saplings and shifts in above and belowground communities caused by *I. glandulifera* may influenced forest function and regeneration after disturbance and reduce timber production. To avoid or reduce such negative effects on forest diversity, it is recommended to remove the invasive plant in the colonising phase, since the initial state of forest habitat can be recovered by removal of *I. glandulifera.*

REFERENCES

Agerer, R (1987-2002) Colour atlas of ectomycorrhizae. München, Einhorn Verlag,

Boag, DA (1990) On the effectiveness of artificial shelters in the study of population attributes of small terrestrial gastropods. Can J Zool 63:1089–1095.

Callaway RM, Aschehough ET (2008) Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science 290:521–523

Cote JF, Thibault JR (1988) Allelopathic potential of raspberry folliar leachates on growth of ectomycorrhizal fungi associated with black spruce. Am J Bot 75:966–970.

Erland S, Talyor ASF (2002.) Diversity of ectomycorrhizal fungal communities in relation to the abiotic environment. Pages 163–200 *in*: van der Heijden MGA, Sanders I (Eds), Mycorrhizal ecology. Ecological Studies. Springer, Berlin, Heidelberg, Germany.

Hawkins JW, Lankester MW, Nelson RRA (1998) Sampling terrestrial gastropods using cardboard sheets. Malacologia 39:1–9.

Inderjit, Bajpai D, Rajeswari MS (2010) Interaction of 8-Hydroquinoline with soil environment mediates its ecological function. PLoS ONE. 5:9 e12852

Lorenzo P, Palomera-Pérez A, Reigosa MJ, Gonzàlez L (2011) Allelopathic interference of invasive *Acacia deabata* Link on the physiolocial parameters of native understory species. Plant Ecol 212:403–412

Mason CR (1970) Snail populations, beech litter production and the role of snails in litter decomposition. Oecologia 5:215–239

Rice EL (1979) Allelopathy: an update. Bot Rev 45:15–109.

Rietveld WJ (1983) Allelopathic effects of juglone on germination and growth of several herbaceous and woody species. J Chem Ecol 9:295–308

Rose SL, Perry DA, Pilz D, Schoeneberger MM (1983) Allelopathic effects of litter on the growth and colonization of mycorrhizal fungi. J of Cheml Ecol 9:1153–1162.

Smith SE, Read SJ (2009) Mycorrhizal symbiosis. 3rd edition. Academic, Amsterdam.

Vaughn SF, Berhow MA (1999) Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). J Chem Ecol 25:2495–2504

Wallwork JA (1976) Ecology of Soil Animals. McGraw-Hill, New York.