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

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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

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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