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Invasion by Fallopia japonica alters soil food webs through secondary metabolites

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ABSTRACT

Biological invasions are a major threat to biodiversity with varying degrees of impact. There is increasing evidence that allelopathy often plays an important role in explaining both invasion success and impact on native taxa (e.g. novel weapons hypothesis). The effects of these secondary metabolites on plant communities and microorganisms are well known. However, their direct and indirect effects on soil fauna are unresolved, despite the importance of the latter in ecosystem processes and, potentially, invasion mitigation. Japanese knotweed (*Fallopia japonica*), an east-Asian species, which has proved to be invasive in Europe, containing allelopathic secondary compounds inhibiting native plants and microbial communities. The focal point of this study was the allelopathic effects of knotweed on soil mesofauna (Nematoda, Collembola and Acari). During a one-month laboratory experiment we added knotweed rhizome extract (KRE) at different concentrations to soils collected in an invasion-prone area. He experiment consisted of including or excluding secondary metabolites through the use of activated carbon filtration of KRE. This enabled us to separate effects caused by nutrient addition (i.e. trophic effects) and combined (trophic and allelopathic) effects. Relative effects of nutrient and secondary metabolites addition on abiotic and biotic soil variables were then quantified. We highlighted frequently contrasting trophic and allelopathic effects influenced in some cases by KRE concentration. Microbial assemblages, through fungal/microbial biomass ratio, did not show any congruent response to KRE secondary compounds but was more negatively impacted by nutrient addition. The use of a trophic-based path analysis led us to show that changes within the soil biota had repercussions on secondary consumers (e.g. bacterivorous nematodes and Collembola). Abundance within taxa at higher trophic levels such as predatory Acari (but not predatory nematodes) was also affected although to a lesser extent, likely in part due to the limited considered timeframe. Overall, we showed that, in controlled conditions, invasive allelopathic plants such as knotweeds can have effects on soil fauna at different trophic levels through addition of both nutrients and secondary metabolites to the soil. Considering the limited knowledge of allelopathic effects on the soil fauna and soil functions, this study provides new information on above- and belowground interactions.

1. Introduction

Past and current introduction of invasive plant species and their spread in new ecosystems is a major concern for conservation at a global level (Litt et al., 2014; Pyšek et al., 2012) due to their severe impact on biodiversity (Murrell et al., 2011; Vilà et al., 2011) and ecosystem processes (Bassett et al., 2011; Kohyt and Skubała, 2013). Only a small number of exotic species become invasive in their introduced range (Reinhart and Callaway, 2006) through distinctive characteristics (or traits) providing superior competitive ability when compared to native species (Van Kleunen et al., 2010). These traits can be morphological in nature by directly improving plant fitness (Van Kleunen et al., 2010) or physiological with the synthesis of biochemical, secondary metabolites that influence the germination, growth, survival and/or reproduction of other organisms (Inderjit et al., 2011b).

The novel weapon hypothesis (NVH) suggests that the success of many exotic invasive plant species is due to the possession of allelopathic compounds unencountered by native species, particularly native plant species (Callaway and Ridenour, 2004). Furthermore, it has been shown that many invasive species have different allelopathic potential effects between their native and introduced ranges (Inderjit et al., 2011a; Thorpe et al., 2009). These biochemical compounds, exudated from plant roots (Callaway et al., 2008) or released from degrading litter (Inderjit et al., 2011a) have powerful effects on ecosystem

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functioning by impacting both organisms and ecological processes (Hättenschwiler et al., 2011; Hättenschwiler and Vitousek, 2000; Reigosa et al., 2006; Wardle et al., 1998).

Under the soil, plants interact with a wide range of organisms including bacteria, fungi, nematodes and various kinds of arthropods (Abgrall et al., 2017; Parepa et al., 2013). These aboveground-belowground relationships can be antagonistic (e.g. herbivores, pathogens) or mutualistic (e.g. mycorrhizeous fungi, nitrogen-fixing bacteria) (Van der Putten et al., 2007). Allelopathic biochemical that have a negative effect on plants can do so indirectly by promoting or inhibiting particular soil biota (Callaway et al., 2008; Stinson et al., 2006). Furthermore, the soil biota is known as having a structuring influence on plant community composition, dynamics and phenology (Forey et al., 2015; Wardle, 2002), allelopathy feedback from the soil biota could further increase invasion (Parepa et al., 2013).

Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decr. 1988, Polygonaceae) was introduced in Europe in the 19th century for its ornamental properties. It is now one of the most destructive invasive species in Europe and North America (Lowe et al., 2000). *F. japonica* spreads mostly by clonal rhizomatous growth with a single stem or rhizome node being able to regenerate a full plant explaining the high-dispersion capacity of knotweed (De Waal, 2001). Multiple species of the genus *Fallopia* such as *F. japonica, F. sachalinensis* as well as their hybrid *F.* × *bohemica* are known to contain and produce several secondary metabolites (Murrell et al., 2011). Some of those compounds exhibit allelopathic properties and can inhibit the germination or growth of other plant species (Aguilera et al., 2010; Gerber et al., 2008) as well as bacteria (Hedenec et al., 2014) with mixed effects on fungi (e.g. Daayf et al., 1995; Kumagai et al., 2005). A study by Vastano et al. (2000) revealed a higher concentration of stilbenes in North American invasive *F. japonica* than in Chinese native individuals of the same species tending to support the NVH in the case of knotweed. One of these compounds, *trans*-resveratrol (3,4,5′-trihydroxystilbene), has been identified as being produced by knotweed (Vastano et al., 2000). This molecule, which is also found in grapevines, is known as having antifungal (Filip et al., 2003) and antibacterial properties (Chan, 2002). Content analysis of resveratrol in knotweed tissues has been assessed by Vaher and Koel (2003) who found that more than 80% of *trans*-resveratrol was located in the roots and rhizomes, where the majority of plant-microorganisms interactions occur (Bais et al., 2006).

Secondary metabolites present in knotweed rhizomes could have either a direct effect on soil fauna either by repellence (Asplund et al., 2015), toxicity (Isman and Duffey, 1982) or an indirect effect through changes in the soil biota (Ens et al., 2009). As evidence for direct toxicity of phenolic compounds is scarce, indirect effects through alterations of basal resources for secondary consumers appear more likely. In this paper, we studied the effect of knotweed rhizome aqueous extracts on the soil biota and fauna in order to provide additional information on the novel weapon hypothesis in this particular case. Indeed, while several studies have assessed knotweed allelopathic potential in invaded areas none, as far as we know, have considered the impact on the soil fauna in relation to this hypothesis. Therefore and based on the theory, we hypothesized that: (1) knotweed has a negative effect on microbial (and particularly bacterial) biomass through rhizome allelopathic secondary metabolites; (2) this negative effect has repercussions on higher trophic levels through trophic cascades, and results in soil food web structure alteration; (3) this negative effect is be slightly attenuated by a positive trophic effect of nutrient addition provided by knotweed rhizome extract (4) those effects, positive (i.e. trophic); or negative (i.e. allelopathic), are concentration-dependent.

2. Material & methods

2.1. Material collection and experiment preparation

Belowground *F. japonica* biomass was harvested in early autumn 2016 within a spontaneously invaded plateau site in Normandy, France (49.455024° N; 1.062645° W). To the best of our knowledge, control measures have never been applied to this site. Samples were kept in an icebox for transportation to the laboratory. Rhizomes were water-cleaned and stored at 4 °C prior to extraction. We used an electric grinder to break plant tissues and facilitate osmosis. One hundred grams of ground plant material was mixed with 1000 ml of distilled water. This aqueous extract was kept at 19 °C for 24 h. Following Norsworthy (2003) the mixture was then passed through a series of sieves ranging from 1000 to 50 μm and then vacuum filtered through standard filter paper (> 20–25 μm). The extract was then further sterilized by filtering through 0.22 μm filter.

We collected soil from the upper 10 cm of a reaped grassland in a small valley. While the area was uninfested by *F. japonica* close-by sites (< 200 m) with similar topographical and edaphic conditions have been invaded for several years. Macrofauna as well as macroscopic plant materials were removed from the collected soil. Samples were gently, and unforcefully, sieved at 5 mm so as to preserve mesofauna and mixed. Ten 200 g samples were taken from the soil to sample initial Collembola, Acari and Nematoda communities. Soils were placed in 8 × 8 × 10 cm plastic pots. Filter paper (< 10–20 μm) was placed at the bottom of the prevent leakage of the pot content. Sixty grams of fine grained sand was added above the filter paper forming a ~0.5 cm layer. The rest of the pot was filled with 310 ± 5 g of soil. Ten 400 g samples of mixed soil were also collected for analysis of initial physico-chemical conditions. A layer of 0.5 g of *Agropyron* sp. litter, which is the dominant species in the samples grassland and also present close to invaded sites, was added to provide physical habitat for the soil fauna. Pots were kept at 19 °C in a phytotron with a 8 h day/16 h night cycle for a week. In order to increase and homogenize abundance and compensate for possible losses during soil sieving each pot was then placed under 2 individual Berlese-Tullgren extractors, one containing topsoil (0–5 cm) and the other deeper soil (5–10 cm) from the same area.

2.2. Experimental design

To simulate varying natural conditions and test for concentration-dependence distilled water was used to provide different concentrations of the aforementioned aqueous KRE (0, 33, 66 & 100%). Half of the solution at each concentration, including distilled water, was filtered 3 times through activated carbon prior to watering. This filtration was conducted in order to remove potentially toxic organic compounds (Cheremisinoff and Ellerbusch, 1978) from the KRE. We also filtered distilled water in order to test for the effect of filtration itself.

In total we obtained 8 different solution: filtered and unfiltered distilled water, filtered and unfiltered 33% KRE, filtered and unfiltered 66% KRE as well as filtered and unfiltered 100% KRE. Each solution was used to water 10 pots prepared as detailed above. The result was a balanced factorial design (4 × 2 x 10 = 80 pots with 10 replicates per modality). During the experiment, the pots were kept for four weeks (from early November to early December 2016) in a climate-controlled room (21.0 ± 1.9 °C, 16 h day/8 h night, 47.0 ± 8.8% humidity) and watered weekly with the corresponding solution.

2.3. Sampling, biochemical analysis and fauna identification

In order to verify the validity of our activated-carbon methodology we used HPLC to test for resveratrol concentration in filtered and unfiltered KRE. Resveratrol ($C_{14}H_{12}O_3$), a phenolic allelopathic compound, was measured using direct-injection high-performance liquid chromatography (ThermoFisher Scientific UltiMate 3000 UHPLC). We used a variable wavelength UV detector at 306 nm, equipped with a standard C18 column, a water-acetonitril (60:40) mobile phase and an isocratic flow of 1 ml min^{-1} (Goldberg et al., 1994). We used commercially-available Resveratrol powder (CAS Number: 501-36-0) for calibration.