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REGULAR ARTICLE

Diversity- and density-mediated allelopathic effects of resident plant communities on invasion by an exotic plant

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Abstract

Aims Resident plants can exert allelopathic effects on introduced exotic plants, and resistance to exotic plant invasions usually increases with diversity and density of the resident plant communities. We hypothesize that allelopathic effects increase with increasing diversity and density of the community, and thereby contribute to the community's resistance against invaders.

Methods To test these hypotheses, we conducted two greenhouse experiments in which we grew five individuals of the exotic invasive plant *Solidago canadensis* in pots without residents and in artificially assembled resident plant communities either with two levels of diversity (4 or 8 resident plant species) or two levels of density (8 or 32 individuals, representing 8 species). We used activated carbon as the allelopathy-neutralizer treatment in both the diversity and the density experiment.

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Results In the absence of activated carbon, *S. canadensis* grew worse in the presence of residents, and when diversity or density of the residents increased. These negative effects, however, largely disappeared in the presence of activated carbon, and at the highest resident density *S. canadensis* even performed better with than without activated carbon.

Conclusions Low invasibility of species-rich and dense plant communities is likely to be at least in part associated with increased allelopathy. Our study provides the first evidence that increasing allelopathy could be a mechanism underlying the diversity and density effects on community invasibility.

Keywords Alien plant · Allelochemicals · Competitive dominance · Complementarity effect · Sampling effect · Selection effect · Species richness

Introduction

Biological invasions have received much consideration due to the potential threats they impose on ecosystem processes and functioning (Pearson et al. 2018; Petruzzella et al. 2018). Successful plant invasions are attributed to the interaction between the exotic plants and the resident plant communities (Gallien and Carboni 2017; Theoharides and Dukes 2007). Exotic invasive plants may outperform native species due to the absence of their natural enemies in the introduced range (Aguilera 2011; Callaway and Ridenour 2004; Keane and Crawley 2002). Moreover, invasive plants are regarded to possess superior characteristics, such as high competitive ability, efficient absorption and utilization of resources (Baker 1965; Levine 2000; Petruzzella et al. 2018; van Kleunen et al. 2015), a ruderal strategy (Guo et al. 2018) and capability of clonal growth (Parsons and Cuthbertson 2001; Song et al. 2013; Wang et al. 2017). A large body of studies has also elaborated on the allelopathic potential of invasive plants, which may reduce competition by resident plants (Callaway and Ridenour 2004; Dostál 2011; Goel et al. 1989; Prati and Bossdorf 2004; Wardle et al. 1998). However, the resident plants may also produce allelopathic compounds, and it could be that allelopathy of resident plants provides resistance against invaders (Weidenhamer and Romeo 2005). Although this idea was already posed by Rabotnov (1982), it has rarely been tested (Cummings et al. 2012; Hou et al. 2012; Ning et al. 2016; van Kleunen et al. 2018).

Another characteristic of the resident community that might provide resistance against invaders is diversity (Elton 1958). Communities with high species diversity can more fully exploit the available resources (e.g. light, water and nutrients) and space due to niche differentiation and complementarity (Dostál 2011; Emery and Gross 2007; Ortega and Pearson 2005; van Ruijven et al. 2003), leaving less resources and space to potential invaders. This is particularly the case if the community includes different functional groups, such as grasses, nitrogen-fixing forbs and non-nitrogen-fixing forbs. Moreover, diversity increases the stability of a community (Pearson et al. 2018), thereby providing fewer windows of opportunity for establishment of invaders. Furthermore, high plant diversity of communities might lead to a complex food web (Emery and Gross 2007; Zak et al. 2003), with a higher stability and lower invasibility compared to less diverse communities (Emery and Gross 2007). In addition to complementarity effects, communities with high plant diversity may also display a distinct sampling (selection) effect (Emery and Gross 2007; Holmes et al. 2016; Wu et al. 2017; Zhu et al. 2015). For instance, inclusion of a highly competitive C4 grass species in a resident community was shown to effectively reduce biomass of invading species (Fargione and Tilman 2005). Another mechanism through which species diversity may reduce invasibility of resident communities is that higher species diversity results in a higher diversity of allelopathic compounds, and stronger allelopathy overall. To the best of our knowledge, this hypothesis has never been tested.

The density of plants in a community also determines competition intensity and the efficiency to exploit available resources and space (Naeem et al. 2000; Teixeira et al. 2017; Turnbull et al. 2010; Wu et al. 2017). Denser communities thus should leave less resources and space for potential invaders (Dostál 2011; Dukes 2002; Michelan et al. 2013). Furthermore, a high degree of canopy closure in dense communities may also effectively inhibit seed germination, and seedling and juvenile plant growth of invasive exotic species (Christina et al. 2015; Emery and Gross 2007; Liao et al. 2015; Pearson et al. 2018). This is also supported by the fact that undisturbed communities with a closed canopy are less likely to be invaded by exotic plants compared to frequently disturbed communities with an open canopy (Arroyo et al. 2018; Davis et al. 2000; Sun et al. 2015; Tan et al. 2015). In addition, the effect of density on the invasibility of resident plant communities may also be due to increased concentrations of allelopathic compounds. To date, however, no study has tested whether denser communities generate stronger allelopathic effects on invasive exotic plants.

To test the hypotheses that allelopathic effects increase with increasing diversity and density of the community, and thereby contribute to the community's resistance against invaders, we conducted two greenhouse experiments using activate carbon to neutralize allelopathic effects. In one experiment, we planted mesocosm pots with resident grassland communities with different levels of species richness, and in the other we planted mesocosm pots with resident grassland communities with different density levels. In both experiments, we also had control pots without resident grassland communities. Then, the pots were invaded by the exotic plant *Solidago canadensis*, which is highly invasive in China. Specifically, we addressed the following main questions. (1) Is the allelopathic effect of the resident plant communities on the growth of *S. canadensis* stronger when species richness of the resident communities is higher? (2) Is the allelopathic effect stronger when the density of the plant individuals in the resident communities is higher?

Materials and methods

Plant species

Solidago canadensis L. (Asteraceae), native to North America, is listed as an invasive weed in many countries (Abhilasha et al. 2008; Zhang et al. 2009). It was introduced to China as an ornamental plant in 1935 and has spread into the wild since 1980 (Li et al. 2001). *Solidago canadensis* can grow over 1.5 m tall, and can reproduce sexually by producing large amounts of seeds. As it produces a branched rhizome system belowground from which multiple erect stems emerge, it can also reproduce asexually. The seeds are small and dispersed by wind. Due to its tall stature and extensive clonal growth, the species shows strong competitive ability and can displace native plant species, especially in habitats disturbed by human activities (Chen et al. 2012; Li et al. 2010). In Europe, it was shown that *S. canadensis* may have allelopathic effects on native plant species (Abhilasha et al. 2008). In the middle of November 2015, seeds of *S. canadensis* were collected in a suburb of Hangzhou, Zhejiang Province, China. On March 1, 2016, seeds of *S. canadensis* were sown in boxes (length: 38 cm; width: 28 cm; height: 14 cm) filled with river sand. On May 2, 2016, seedlings of similar size were selected for use in the two experiments described below.

We used a total of eight species that are commonly found in grasslands in China to construct resident plant communities. To increase functional diversity and have realistic communities, we chose the eight grassland species in such a way that they belonged to three different functional groups, i.e. four grasses (*Festuca elata* Keng ex E. Alexeev, *Poa pratensis* L., *Bromus inermis* Leyss and *Lolium perenne* L.), two legume forbs (*Trifolium pratense* L. and *Trifolium repens* L.) and two non-legume forbs (*Cichorium intybus* L. and *Ixeridium sonchifolium* (Maxim.) Shih). It should be noted that the two *Trifolium* species are not native to China, but have been present there for at least two centuries (http://www.chinaias.cn/wjPart/SpeciesSearch.aspx?speciesType=3), last visited on April 5, 2019) and are now so common in China that they are part of many resident grassland communities. To make the results more representative, we did not a priori select resident species with known allelopathic effects. However, a posteriori literature searches revealed that at least one of the species, *T. pratense*, has known allelopathic effects (Liebman 2006; Lou et al. 2016). The seeds of the eight species were bought from Beijing Zhongshu Dasen Seed Co. Ltd. in Beijing, China. On April 1, 2016, one month after sowing the seeds of *S. canadensis*, seeds of the eight grassland species were sown in boxes (length: 38 cm; width: 28 cm; height: 14 cm) filled with river sand. All eight species successfully germinated within one week.

Experimental design

We conducted two greenhouse experiments to test the effects of species diversity and density of plant individuals, respectively, on allelopathic potential of the resident plant communities against the invasive herb *S. canadensis*.

Species-diversity experiment: To test the effect of species diversity on allelopathic potential against *S. canadensis*, we carried out an experiment with three levels of resident-species diversity (0, 1 or 4 plant species) crossed with two levels of activated carbon addition (with or without). For the 0-, 1- and 4-species treatments, each pot (23 cm in diameter and 22 cm in height) was planted with no seedlings of resident species, 12 seedlings of the same resident species or 12 seedlings of four resident species (each species having three seedlings) surrounding the central area where seedlings of the invasive species *S. canadensis* were planted (Appendix Fig. 1). The 12 seedlings were arranged at equal intervals in two circles around the central area of the pot, with eight seedlings in the outer circle and four seedlings in the inner circle (Appendix Fig. 1). For the 4-species treatment, each species occurred twice in the outer circle and once in the inner circle, and seedlings of the same species were not adjacent (Appendix Fig. 1). The four species used in this experiment were *B. inermis*, *L. perenne*, *T. repens* and *C. intybus*. Activated carbon can absorb allelopathic substances from plant roots and thereby supposedly neutralizes the allelopathic effects (Lau et al. 2008; Weißhuhn and Prati 2009). For half of the pots, we mixed the soil with 20 ml L^{-1} activated carbon (CASNO: 7440-44-0, Tianjin Woersi Chemical Corporation), and for the other half no activated carbon was added. The amount of activated carbon added followed Lau et al. (2008) and Weißhuhn and Prati (2009). The soil was a 1:1 mixture of quartz sand and vermiculite, with 3.5 g L^{-1} slow-release fertilizer (Osmocote, N:P:K:MgO = 16:9:12:2). We used a non-organic substrate to reduce the side effect of activated carbon on soil nutrient release from organic matter, as it has been reported that the addition of activated carbon could trigger organic nutrient release (Weißhuhn and Prati 2009). On April 23, 2016, plants of the four resident species were transplanted into the pots. One week later, on May 2, 2016, five seedlings of *S. canadensis* were transplanted into the centre of each pot. Initial height of the *S. canadensis* seedlings was 0.91 ± 0.02 cm (mean ± SE). Each species and diversity level were replicated six times, making a total of 72 pots, i.e. [1 (0-species) + 4 (1-species) + 1 (4-species)] × 2 (activated carbon) × 6 (replications).

Density experiment: To test the effect of plant density, we conducted an experiment with three levels of resident-plant density (none, low density and high density) crossed with two levels of activated carbon addition (with or without). In the 'none' level, no resident plants were grown. In the low- and high-density levels, each pot (23 cm in diameter and 22 cm in height) was planted with 8 and 32 seedlings of the eight resident grassland species, respectively. Thus, each of the low- and high-density pots had eight resident species, each with 1 or 4 seedlings, respectively. In half of the pots, we had mixed the soil with 20 ml L^{-1} activated carbon (CASNO: 7440-44-0) and in the other half, no activated carbon had been added. The substrate used was the same as the one used in the species-diversity experiment. On April 23, 2016, seedlings of the eight resident species were transplanted into the pots. On May 2, 2016, five seedlings of *S. canadensis* were transplanted into the center of each pot. Initial height of these seedlings was 0.96 ± 0.02 cm (mean ± SE). Each treatment was replicated six times, resulting in a total of 36 pots.

Both experiments were carried out in a greenhouse of Beijing Forestry University, China (40°40′32″ N, 116°20′24″ E). As all pots were placed within a small area where spatial heterogeneity in microclimatic conditions was expected to be minimal, we re-arranged the pots randomly only twice during the ten weeks of the experiments. Plants were watered daily to keep the soil moist. During the whole period of the experiment, daily mean temperature in the greenhouse was 24.8 ± 0.5 °C (SE), and light transmittance into the greenhouse was c. 60%. The average light period was 14.5 h per day; no additional lighting was provided. No plants of *S. canadensis* died during the experiment, and the resident plants that died during the first week were replaced immediately.

Measurements and harvest

On July 2, 2016, in each pot we recorded the height of each *S. canadensis* plant to calculate the mean height. We harvested the roots, stems and leaves separately for the five *S. canadensis* plants in each pot. Total leaf area of *S. canadensis* was obtained using WinFOLIA Pro 2004a (Regent Instruments, Inc., Canada). Aboveground and belowground biomass of the resident plant community was also harvested. All samples were dried at 70 °C for at least 72 h and then weighed.

Statistical analyses

We used two-way ANOVAs to test the effects of activated carbon addition (with vs. without) and resident-community presence & richness (0 vs. 1 vs. 4 resident plant species) for the species-diversity experiment or resident-community presence & density (0 vs. 8 vs. 32 resident plant individuals) for the density experiment on the growth (total biomass, root biomass, stem biomass, leaf biomass, height and total leaf area) of the invasive plant *S. canadensis*. For the species diversity experiment, following ANOVA, we further used two planned contrasts to separate the effect of resident-community presence & richness into the effect of resident-species presence [0 species vs. (1 species + 4 species)] and the effect of resident-species richness (1 vs. 4 species) (Sokal and Rohlf 1995). We also did two planned contrasts to separate the activated carbon addition × resident-community presence & richness interaction into the activated carbon addition × resident-species presence interaction and the activated carbon addition × resident-species richness interaction (Sokal and Rohlf 1995). Similarly, for the density experiment, we used two planned contrasts to separate the effect of resident-community presence & density into the effect of resident-species presence [0 individuals vs. (8 individuals + 32 individuals)] and the effect of resident-individual density (8 vs. 32 individuals) and also two planned contrasts to separate the activated carbon addition × resident-community presence & density interaction into the activated carbon addition × resident-species presence interaction and the activated carbon addition × resident-individual density interaction. All data were transformed to $\log_{10}$ to increase normality and/or homogeneity of variance. SAS 9.1 was used for all the analyses (SAS Institute Inc. 2004).

Results

Effects of resident-plant species diversity on invasive plant growth

The presence of resident plant species markedly decreased all growth measures (total, root, stem and leaf biomass, height and total leaf area) of the invasive plant *S. canadensis* (Fig. 1, Table 1: effect of resident presence). Increasing species richness (from 1 to 4 species) of the resident plant community decreased total biomass, root biomass, leaf biomass and total leaf area of *S. canadensis* without activated carbon addition, but tended to increase the values of these traits or had no impact with activated carbon (Fig. 1, Table 1: effect of activated carbon addition × resident richness). Averaged across all diversity treatments, activated carbon addition significantly decreased biomass and height, and marginally significantly decreased total leaf area of *S. canadensis* (Table 1, Fig. 1). However, this negative effect of activated carbon addition on total biomass, root biomass, leaf biomass and total leaf area was stronger when the resident community consisted of 1 than of 4 species (Fig. 1), as indicated by the significant or marginally significant interaction effect of activated carbon addition × resident species richness (i.e. A × RR, Table 1).

Effects of resident-plant individual density on invasive plant growth

Compared to the absence of resident plants, the presence of resident plants significantly decreased all growth measures of the invasive plant *S. canadensis* in the absence of activated carbon, but had little impact in the presence of activated carbon addition (Fig. 2, Table 2: main effect of resident presence and effect of activated carbon addition × resident presence). The growth of *S. canadensis* was also significantly lower when the resident community consisted of 32 instead of 8 plant individuals without activated carbon addition, but such effects diminished with activated carbon addition (Fig. 2, Table 2: effect of activated carbon addition × resident density). The effect of activated carbon addition on the growth of *S. canadensis* depended on whether the resident plant community was absent or present (Table 2: effect of activated carbon addition × resident presence) and also on the density of the resident plant communities (Table 2: effect of activated carbon addition × resident density). The effect of activated carbon addition was more negative when the resident plant community was absent than when it consisted of 8 plant individuals, and became positive when it consisted of 32 individuals (Fig. 2).

Discussion

It has long been shown that species-rich communities and undisturbed communities with closed, dense vegetation are less prone to exotic plant invasions (Callaway et al. 2005; Elton 1958; Naeem et al. 2000; Vojik and Boublik 2018). Several hypotheses have been proposed to explain such diversity and density effects on the invasibility of resident plant communities; for instance, species-richer or dense communities can make better use of resources and thus leave less resources for invasive species (Fargione and Tilman 2005; van Ruijven et al. 2003; Zhu et al. 2015). We also found that species-

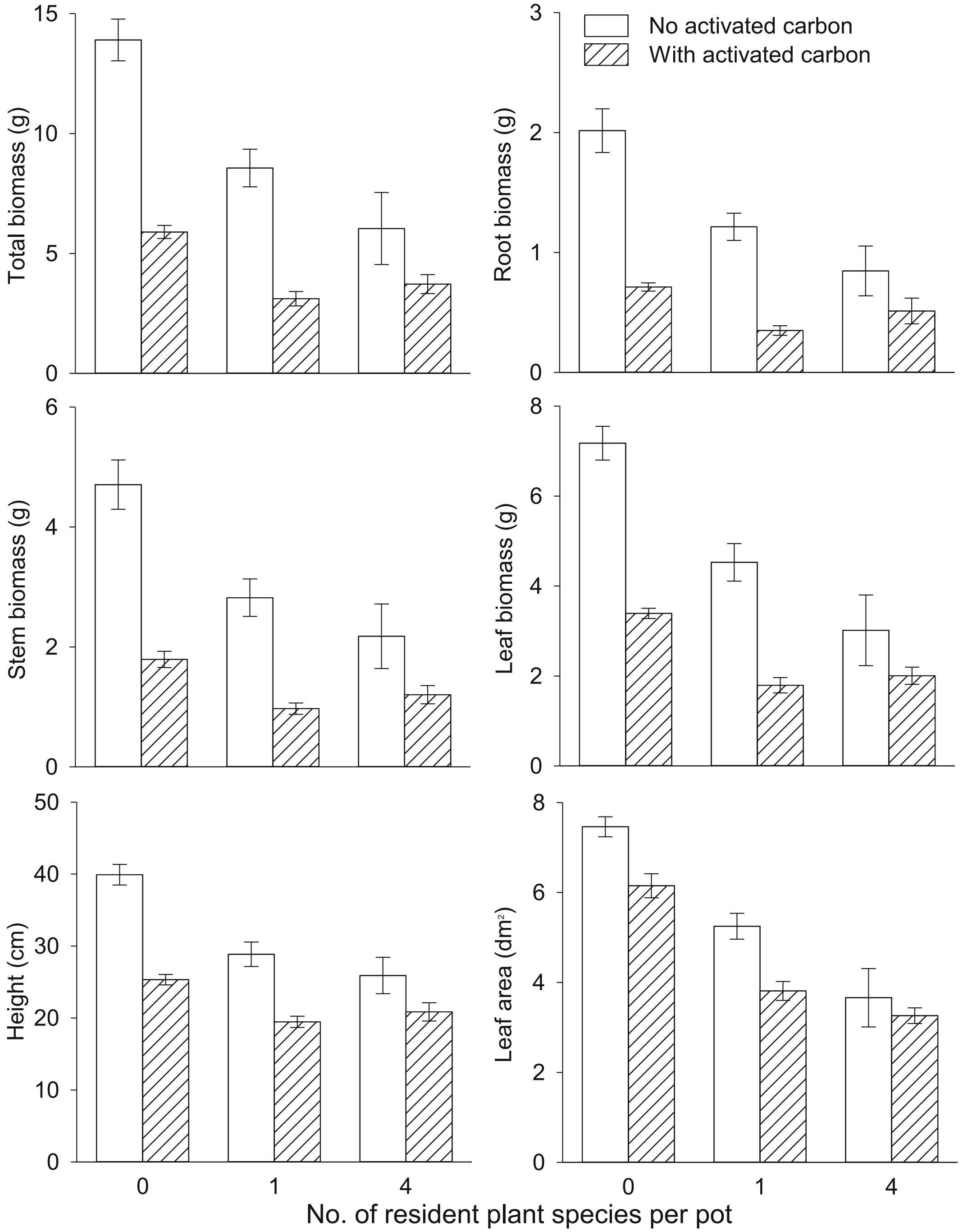

Fig 1 Biomass, height and total leaf area of *Solidago canadensis* growing alone or in the resident communities with two levels of species diversity with or without activated carbon. Mean ± SE are given

diversity and density provided resistance against invasion by *S. canadensis*. However, these effects largely disappeared when activated carbon was mixed into the soil. Although activated carbon may have other effects besides adsorption of allelochemicals (Kabouw et al. 2010; Lau et al. 2008; Wurst et al. 2010), and could also remove allelopathic effects of the invader on the residents as well as autotoxicity of the plants, our results could indicate that increasing species diversity and plant density may increase the allelopathic effects of resident

Table 1 Results of ANOVAs for effects of activated carbon addition (A) and resident community presence & richness (R, i.e. 0 vs. 1 vs. 4 plant species) on the growth of *Solidago canadensis*

Effect	DF	Total biomass	Root biomass	Stem biomass	Leaf biomass	Plant height	Leaf area
Activated carbon (A)	1, 66	**23.60*****	**35.50*****	**23.84*****	**15.27*****	**17.65*****	*3.63*$^{\#}$
Resid. presence & richness (R)	2, 66	**9.49*****	**9.80*****	**7.57****	**9.40*****	**7.06****	**18.46*****
Resident presence (RP)	1, 66	**18.53*****	**17.95*****	**13.76*****	**18.77*****	**12.85*****	**35.17*****
Resident richness (RR)	1, 66	0.38^{ns}	<0.01^{ns}	<0.01^{ns}	1.26^{ns}	<0.01^{ns}	**8.99****
A × R	2, 66	*2.53*$^{\#}$	**3.48***	1.32^{ns}	*2.65*$^{\#}$	0.63^{ns}	1.49^{ns}
A × RP	1, 66	0.39^{ns}	0.32^{ns}	0.42^{ns}	0.39^{ns}	0.91^{ns}	0.05^{ns}
A × RR	1, 66	**5.06***	**6.90***	2.60^{ns}	**5.31***	0.72^{ns}	*2.90*$^{\#}$

The effect of R was further separated into the effect of resident-species presence [RP, i.e. 0 species vs. (1 species + 4 species)] and the effect of resident-species richness (RR, i.e. 1 vs. 4 species) by two planned contrasts. Similarly, the effects of A × R was further separated into the effect of A × RP and the effect of A × RR by two planned contrasts

F values, degree of freedom (DF) and significance levels (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\#}$ $P < 0.1$ and ^{ns} $P > 0.1$) are given. Values are in bold when $P < 0.05$ and in italics when $0.05 < P < 0.1$. All data were transformed to $\log_{10}$ before analysis

plant communities on the growth of invasive plants. Thus, increasing allelopathy could be an additional mechanism underlying the negative effects of diversity and density of resident communities on their invasibility.

Many ecological studies on the role of allelopathy in competitive interactions used, like we did, activated carbon as an allelopathy neutralizer (Inderjit and Callaway 2003; Prati and Bossdorf 2004; Ridenour and Callaway 2001). Activated carbon, however, can also have side effects on soil properties, microbes and plant growth (Kabouw et al. 2010; Lau et al. 2008; Wurst et al. 2010). Here, we also found evidence for such undesired side effects, as activated carbon reduced the growth of *S. canadensis* plants when grown in the absence of resident plants. This could, however, also indicate that *S. canadensis* had positive allelopathic effects on its own growth, and that these were neutralized by activated carbon. Nevertheless, evidence for its allelopathy neutralizing effect is provided by the finding that activated carbon severely reduced the negative effect of the residents on the growth of *S. canadensis*. In the species-diversity experiment, we cannot exclude that this is due to negative side effects of activated carbon on the growth of the residents (Appendix Fig. 2), which might have reduced their competitive effects on *S. canadensis*. However, in the density experiment this is an unlikely explanation, because in that experiment there was no effect of activated carbon on the growth of the residents (Appendix Fig. 3). Nevertheless, activated carbon reduced the negative effect of the residents, and at the highest density even promoted the growth of *S. canadensis*. Therefore, we conclude that despite its potential side effects, activated carbon most likely acted as an allelopathy neutralizer.

Effects of resident plant diversity on invasive plant growth

Activated carbon addition decreased the growth of the invasive plant species *S. canadensis* in the absence of the resident plant community. However, in the presence of a resident community, the negative effect of activated carbon became weaker when the resident community consisted of four instead of one plant species (Fig. 1). This result provides support for the idea that resident plant communities could impose allopathic effects on exotic plants, as was also suggested by previous studies (Cummings et al. 2012; Hou et al. 2012; Ning et al. 2016; Weidenhamer and Romeo 2005). Moreover, this result supports our first hypothesis stating that with increasing species diversity of the resident plant community, the allelopathic inhibitory effect on the growth of invaders increases.

While at large spatial scales areas rich in native species usually also have more invasive species (Stohlgren et al. 2003), at small spatial scales most studies have shown that increasing species diversity can increase the ability of resident plant communities to resist exotic plant invasions (Petruzzella et al. 2018; Turnbull et al. 2010; Wu et al. 2017). One potential mechanism for the latter is that increasing plant-species diversity can increase resource-use efficiency and thus result in a more complete exploitation of

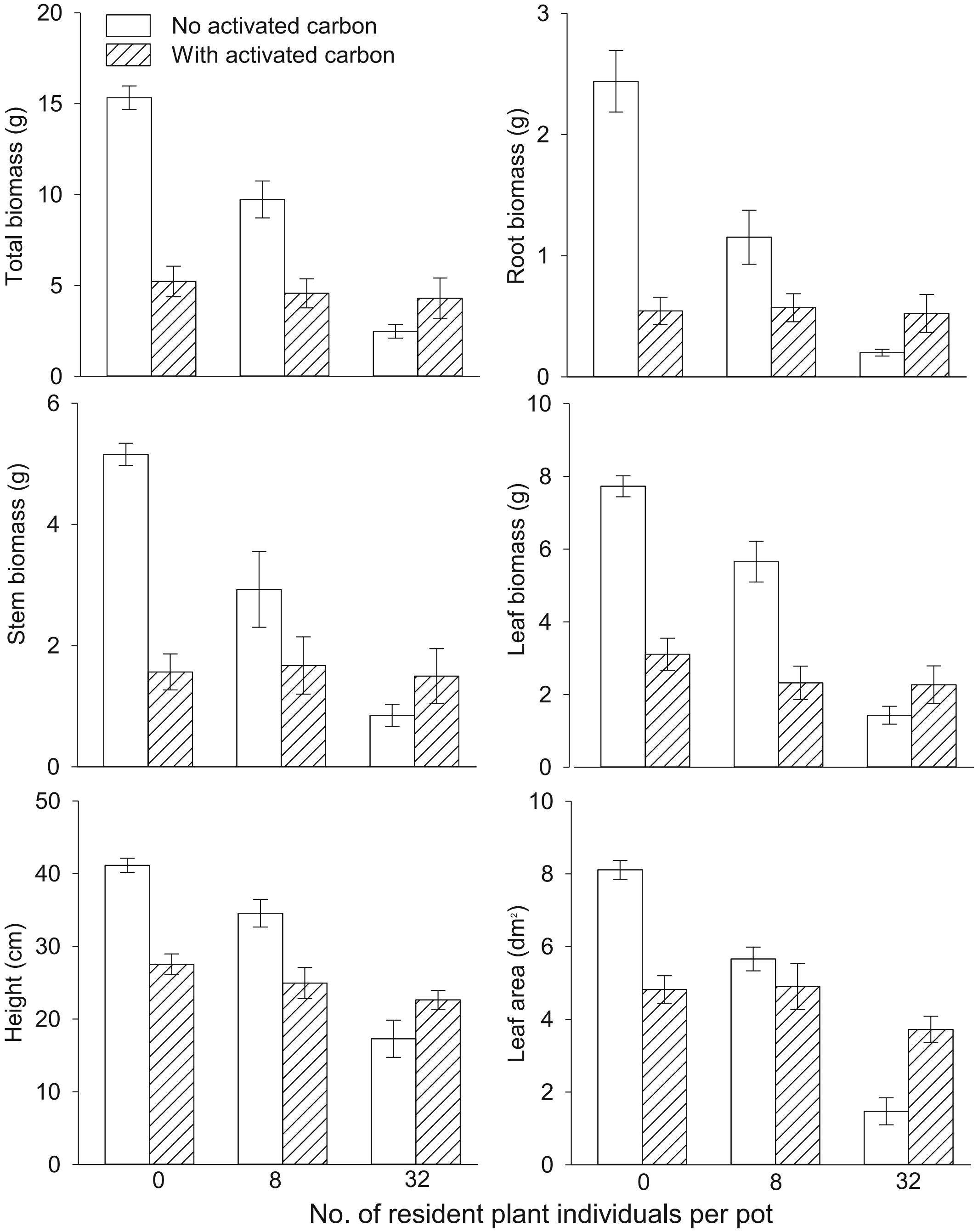

Fig. 2 Biomass, height and total leaf area of *Solidago canadensis* growing alone or in the resident communities with two levels of plant density with or without activated carbon. Means ± SE are given

resources through complementarity effects (Davis et al. 2000; Dostál 2011; Emery and Gross 2007; Fargione and Tilman 2005; Grace et al. 2017). This would leave fewer or lower amounts of resources to support the establishment and growth of introduced plants (Davis et al. 2000). Another potential mechanism is that more diverse plant communities may have a higher chance to include at least one plant species with a high competitive ability to effectively suppress the introduced plant species (i.e. a sampling effect) (Petruzzella et al. 2018; Qin et al. 2013; Wardle 2001). One particular example of this mechanism is that species-richer communities may

Table 2 Results of ANOVAs for effects of activated carbon addition (A) and resident-community presence & density (R, i.e. 0 vs. 8 vs. 32 plant individuals) on the growth of *Solidago canadensis*

Effect	DF	Total biomass	Root biomass	Stem biomass	Leaf biomass	Plant height	Leaf area
Activated carbon (A)	1, 30	**14.12*****	**6.65***	**6.07***	**15.65*****	*3.90*$^{\#}$	2.24^{ns}
Resident presence & density (R)	2, 30	**23.63*****	**17.49*****	**10.33****	**25.60*****	**23.06*****	**28.62*****
Resident presence (RP)	1, 30	**24.92*****	**19.35*****	**12.59****	**27.99*****	**22.57*****	**28.33*****
Resident density (RD)	1, 30	**22.34*****	**15.64*****	**8.08****	**23.21*****	**23.55*****	**28.91*****
A × R	2, 30	**12.89*****	**14.16*****	**6.61****	**12.97*****	**11.05*****	**10.57*****
A × RP	1, 30	**11.04****	**18.03*****	**8.48****	**6.40***	**7.32***	**13.75*****
A × RD	1, 30	**14.73*****	**10.29****	**4.73***	**19.54*****	**14.77**	**7.39***

The effect of R was further separated into the effect of resident-species presence [RP, i.e. 0 individuals vs. (8 individuals + 32 individuals)] and the effect of resident-plant density (RD, i.e. 8 vs. 32 individuals) by two planned contrasts. Similarly, the effects of A × R was further separated into the effect of A × RP and the effect of A × RD by two planned contrasts

F values, degree of freedom (DF) and significance levels (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{\#}$ $P < 0.1$ and ^{ns} $P > 0.1$) are given. Values are in bold when $P < 0.05$ and in italics when $0.05 < P < 0.1$. All data were transformed to $\log_{10}$ before analysis

have plant species that are functionally similar to the invasive plant species, which can greatly enhance the ability of the community to resist exotic plant invasion due to niche overlap (Burke and Grime 1996; Fargione and Tilman 2005; Marraffini and Geller 2015).

It is must be noted that, like any experiment, we cannot extrapolate our results beyond the conditions that we used. For example, soil physico-chemical properties, microbial composition, and propagule pressure of invaders could greatly influence allelochemical interactions among resident and invading species (Inderjit 2001; Schmidt and Ley 1999). Nevertheless, our results suggest that, in addition to the abovementioned complementarity and sampling effects (Fargione and Tilman 2005; Frankow-Lindberg 2012; Levine et al. 2004), increasing species diversity may increase the complementary or the selection effect of allelopathy. In other words, more diverse communities may produce stronger allelopathic effects due to mixing of different allelopathic chemicals produced by different plants or due to the production of a very strong allelopathic chemical by at least one of the species. Indeed, there is evidence that mixtures of different chemicals (of a single species) have stronger negative effects on plant growth than each of the single chemicals (Einhellig 1995; Inderjit and Duke 2003). It is also reasonable to assume that more diverse communities have a higher chance to include species that produce chemicals that have a stronger allelopathic effect (Byun and Lee 2017; Hector et al. 2001; Qin et al. 2013). Therefore, increasing allelopathy with increasing species richness, either due to complementarity or sampling might be one of the mechanisms underlying the finding that species-rich communities are usually more resistant to invasions.

Effects of resident plant density on invasive plant growth

In nature, plant density can vary greatly between different environments, and what is a high density in a temperate, fertile meadow can be quite different from what is a high density in an arid, sandy soil. Not surprisingly, however, we found that with a relative increase in density of the resident community, the growth of the invasive *S. canadensis* decreased. Most other studies on this topic also revealed a negative relationship between invasibility of plant communities and their density, degree of canopy closure or biomass production (Brown and Fridley 2003; Kempel et al. 2013; Vojik and Boublik 2018). Dense communities leave little space and few resources to support additional plant recruitment, and it then obviously becomes difficult for exotic plants to establish. This principle is also well known from agriculture, where studies have demonstrated that there is a positive linkage between high crop density and weed suppression (Parker and Riches 1993; Tollenaar et al. 1994).

Besides the aforementioned mechanisms, increasing plant density of the resident communities may result in a proportional increment of allelopathy. Indeed, like in the diversity experiment, the density experiment also showed a reduced negative effect of activated carbon addition in the presence compared to in the absence of resident plants. This again suggests that the resident plant communities imposed an allelopathic effect on the invasive plant *S. canadensis.* Moreover, the effect of activated carbon addition on the growth of *S. canadensis* switched from negative in the low-density community to positive in the high-density community, implying that the allelopathic effect of the resident plant community became stronger with increasing plant density. These results thus support our second hypothesis that with increasing density of the resident plant community its allelopathic inhibitory effect on the growth of *S. canadensis* increased.

Conclusions

Our study provides novel evidence that increasing allelopathy can be a mechanism underlying the diversity and density effects on community invasibility. The design of our species-diversity experiment, however, does not allow us to tell whether the increased allelopathic effect was due to the mixing of different allelopathic chemicals produced by different species (i.e. a complementarity effect) or due to the production of a stronger allelopathic chemical by at least one specific plant species (i.e. a sampling effect). Future studies should be designed to separate such effects. For example, instead of using activated carbon as an allelopathy neutralizer, studies could add allelopathic chemicals (e.g. Weidenhamer et al. 1989) or mixtures thereof. Moreover, it is somewhat elusive to translate the results from a growth study in a simulated plant community to the natural condition where exotic species have to establish and spread (and have an impact) before they are considered invasive. Therefore, longer-term field studies are needed to test whether allelopathy can be a major mechanism of resistance against exotic invaders. Our study shows that the potential is there, and that it may increase with diversity and density of the resident community.

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Authors' contributions FHY and MvK conceived the ideas and designed methodology; LN collected the data; FHY and LN analysed the data; MOA and MT led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility After acceptance, all data will be deposited at Dryad Digital Repository.

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