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Abundance and Diversity of Seedlings of the Soil Seed Bank in the Thickets of the Invasive Species *Acer negundo* L.

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Abstract—We investigated the assumption of transformation of soil seed banks under the influence of invasive plants. For this purpose, we analyzed the taxonomic diversity and abundance of seedlings from the soil seed bank in the thickets of the invasive ash-leaved maple *Acer negundo* L. We performed our experiments using the seedlings emerged from soil seed banks collected in two types of habitats in Yekaterinburg (dense thickets of *A. negundo* and habitats with similar geomorphological and edaphic features but without *A. negundo*). In addition, we analyzed the seedlings emerged from sod-podzolic soils collected from suburban meadow areas. We observed a small negative effect of *A. negundo* on the abundance of seedlings from the soil seed bank. The amount of seedlings on the soil from the thickets of *A. negundo* was lower by 1.5–2.5 times than in urban habitats without this species. The taxonomic diversity of seedlings differed between suburban and urban habitats but did not depend on the presence of habitat transformation by *A. negundo*.

Keywords: Acer negundo L., ash-leaved maple, biological invasions, invasive plants, soil seed bank
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INTRODUCTION

Among 3.9% of plant species on Earth that have naturalized in new areas (Kleunen et al., 2015), the species that can transform habitats and inhibit normal succession processes are the most dangerous for the diversity of native communities (Vinogradova et al., 2010; Richardson and Pyšek, 2012; Gioria and Osborne, 2014; Kumschick et al., 2015; Gusev et al., 2017). Numerous studies have been performed to estimate the specificity of the effects of invasive species on native species and communities and to determine whether these effects are based on normal ecological processes and phenomena, such as spatial and food competition, dominance, and edificator composition. (Gioria and Osborne, 2014).

The seed soil banks were subjects of numerous studies dedicated to the problem of plant invasions (Vila and Gimeno, 2007; Gooden and French, 2014). The meta-analysis of such studies performed in 2014 included 31 publications (Gioria et al., 2014). The comparison of seed banks under the canopy of 18 invasive species and in control communities showed that the diversity and abundance of seed banks is reduced in presence of foreign species, but the effect can be different depending on the invasive species and the type of community. There was no study performed for ash-leaved maple *Acer negundo* L. included in this review (Gioria et al., 2014); the invasion of only three woody plant species was studied—*Acacia dealbata* Link, *Acacia saligna* (Labill.) H.L. Wendl., and *Eucalyptus cladocalyx* F. Muell.

Our study was aimed to investigation of *A. negungo*, that became common in Europe and Asia and included in the Black Book of Flora of Central Russia (Vinogradova et al., 2010) that transforms native habitats and reduces their diversity (Ryabinina and Nikitina, 2009; Kostina et al., 2015; Gusev, 2016). The purpose of this study is to estimate the taxonomic diversity and abundance of seedlings from the soil seed bank in the monospecific stands of *A. negundo* L. Supported by the results of the cited meta-analysis (Gioria et al., 2014), we suppose that the amount of viable seeds in the thickets of this invasive species and their

Table 1. Parameters of sites and habitats

Parameter	Urban areas						Countryside meadow
	A		B		C		
	A.n.+	*A.n.*–	*A.n.*+	*A.n.*–	*A.n.*+	*A.n.*–	
Location							
N	56°52′58″		56°45′44″		56°48′16″		56°40′18″
E	60°42′13″		60°36′46″		60°38′50″		60°28′12″
Height ASL, m	275		267		238		306
Tree species	*Acer negundo*	–	*Acer negundo*	*Pinus sylvestris* L., *Populus* × *sibirica* G.V. Krylov & G.V. Grig. ex A.K. Skvortsov	*Acer negundo*	*Populus* × *sibirica* G.V. Krylov & G.V. Grig. ex A.K. Skvortsov	–
Height of crowns, m	10–12	–	8–10	20–25	5–7	20–22	–
Projective cover							
crowns	90–100	–	70–90	20–30	100	40–50	–
herbal layer	10–20	70–100	1–5	80–90	1–5	30–70	100
anthropogenic garbage	10–20	10–20	20–30	5–10	20–30	5–10	0
Amount of herbaceous species on an area of 10 × 10 m	6	15	8	14	2	10	29

species diversity are lower than in habitats with similar geomorphological and edaphic features but without this species.

MATERIALS AND METHODS

Model species. This study is supplemental to the experiment conducted by the authors in order to investigate the assumption of an indirect allelopathic effect of invasive *Acer negundo* on the development of two native herbaceous species: *Festuca rubra* L. (red fescue) and *Trifolium repens* L. (white clover). These are typical perennial herbaceous ruderal-meadow species native to meadows and habitats with anthropogenic changes (Tret'yakova, 2011).

Design of experiment. The vegetation experiment included the use of *F. rubra* and *T. repens* which cultivated on soils from three pairs of plots (A, B, and C) in Yekaterinburg. We collected the upper layer of 20 cm from the soil profile in the first ten days of July in two habitats in each area. The test variants (*A.n.*+) of samples were obtained from plots with *A. negundo* thickets. The control variants (*A.n.*–) of samples were obtained outside such monospecific stands. The control and test plots had identical geomorphological and edaphic features (Table 1); the distance between the plots within each area was 20 m or less. All urban plots were located in middle parts and bottoms of slopes; these plots were covered by urban soils with various levels of disproportion of upper horizons. The tree layer in all variants of *A.n.*+ consisted of *A. negundo*; the variants of *A.n.*– were both with and without the tree layer. We also used a negative control: a sod-podzolic soil from a suburban secondary upland meadow, which was located in the lower part of a slope and which had been previously tilled.

Thus, we estimated the growth of model plants in seven variants of soils: three urban sites with two habitats each (with and without *A. negundo*) and one site with one habitat without *A. negundo* outside the city. Each soil variant consisted of six vegetation containers: three containers with the seeds of *F. rubra* and three containers with the seeds of *T. repens*. The total number of containers was 42 (7 soil variants × 2 model species × 3 replicates). During the analysis of data, we divided 42 replicates into three groups: (1) *A.n.*+: 18 containers with soil from urban thickets of *A. negundo*; (2) *A.n.*–: 18 containers with soil from urban sites without *A. negundo*; (3) meadow: 6 containers with soil from a meadow located in non-urban area.

The size of all containers was 25 × 40 × 10 cm. Each of them contained 7–8 L of soil. The soil was sieved before being placed in the containers (mesh size: 0.5 × 0.8 cm). The seeds of *F. rubra* and *T. repens* were sown 100 pcs per each container. The plants were cultivated in a polycarbonate greenhouse for 50 days (from July 20 to September 8, 2016). The containers

Table 2. Results of three-way ANOVA of the number of taxa and seedlings in containers (*F* is *F*-test; *p* is the significance level; *dF* is the number of degrees of freedom)

Source of variability	*dF*	Taxa in containers		Margalef index		Samples in containers	
		F	*p*	*F*	*p*	*F*	*p*
Variant [1]	2	**28.22**	**<0.0001**	**29.34**	**<0.0001**	**3.92**	**0.0242**
Model species [2]	1	0.42	0.5200	0.27	0.6062	0.17	0.6801
Tour [3]	1	0.03	0.8540	**4.16**	**0.0451**	**8.09**	**0.0058**
[1] × [2]	2	0.09	0.9183	0.13	0.8788	0.29	0.7463
[1] × [3]	2	**3.34**	**0.0410**	2.31	0.1069	0.48	0.6199
[2] × [3]	1	0.85	0.3588	1.07	0.3038	0.00	0.9844
[1] × [2] × [3]	2	1.88	0.1605	1.36	0.2635	0.04	0.9602

Significant effects are highlighted in bold.

were watered so that the humidity in most of the variants were 60% of the maximum field water capacity.

Inventory of seedlings emerged from the soil seed bank. The inventory consisted of two steps: one on day 24 (tour I) and the other on day 50, i.e., at the end of the experiment (tour II). During each tour, all the plants (seedlings and plants of older age groups) obtained from the seeds contained in soils were weeded out and herborized. The species were determined using guides (Golubintseva and Lebedev, 1959; Vinogradova, 1984; Vasilchenko, 2012). Some samples were determined only to their genera. Such samples were counted as a separate taxon during analysis.

Data analysis. For the analysis of data, we used ANOVA followed by pairwise estimation of differences by Tukey's test (STATISTICA 10.0; StatSoft, United States). The values of seedlings were transformed into logarithmic form; the unit used in ANOVA was the mean value for all replicates (per container). The correlation of variables was estimated using the Pearson correlation coefficient. The rarefaction curves were obtained during the assessment of total species diversity in EstimateS 9.0.1 (Colwell, 2013). We used the interpolated estimates of the cumulative amount of taxa in variants *A.n.*+ and *A.n.*– and extrapolated estimates for the meadow variant.

RESULTS

In all, in tour I of the inventory, plants of 26 taxa were registered; in tour II, 37 taxa. The main number of seedlings in both tours of the inventory consisted of common species and genera of herbaceous ruderal plants. In half or more of the containers, the following plants were present: in tour I, *Carum carvi* L., *Chenopodium album* L., *Plantago* spp., *Taraxacum officinale* F.H. Wigg., *Urtica* spp.; in tour II, *Chenopodium glaucum* L., *Epilobium* spp., *Plantago* spp., *Poa annua* L., *Potentilla supina* L., *Urtica* spp. Since many specimens were not able to be determined to the level of species, we did not examine the taxonomic or other structures of communities of the seed bank, but analyzed attributes of diversity and abundance of seedlings.

Taxonomic diversity. The amount of seedlings in different containers varied from 2 to 10 in tour I and from 2 to 12 in tour II. The mean number of taxa in each container varied significantly only between the variants and did not differ between the model species (*F. rubra* or *T. repens*) or the tours (Table 2). Highest abundance (8–9 taxa per container) was observed in the countryside meadow area (Fig. 1a). The lowest abundance (4–6 taxa per container) was observed in urban soils regardless of *A. negundo* presence. Differences in the abundance of seedlings between variants were significant only between the meadow and urban variants ($p = 0.0001$ according to Tukey's test).

The Margalef index of species diversity and adjusted estimates of the number of taxa per counted samples varied in the same way as the number of species per container. The diversity of seedlings depended only on the variant and did not depend on the model species or on the tour. The values of the Margalef index were predictably high in the meadow area (2.30–2.79) and low in the urban areas (1.12–1.58). According to Tukey's test, differences between the meadow and urban areas were significant ($p = 0.0001$).

Abundance. The absolute amount of seedlings in different containers varied from 5 to 99 in tour I and from 3 to 96 in tour II. The mean number of samples per container varied significantly depending on the variant and on the tour but did not depend on the model species. The abundance of seedlings was higher in tour I as compared with tour II, but in both tours, it was the highest in urban areas without *A. negundo* and the lowest in the monospecific stands of *A. negundo* (Fig. 1b). According to Tukey's test, differences between the *A.n.*+ and *A.n.*– were significant ($p = 0.0203$).

Consistency of abundance and diversity estimates between the tours. The consistency of variability patterns of abundance and diversity in both tours indicates that changes in these parameters are somehow related to each other. The correlation coefficient between the amounts of taxa in containers recorded in

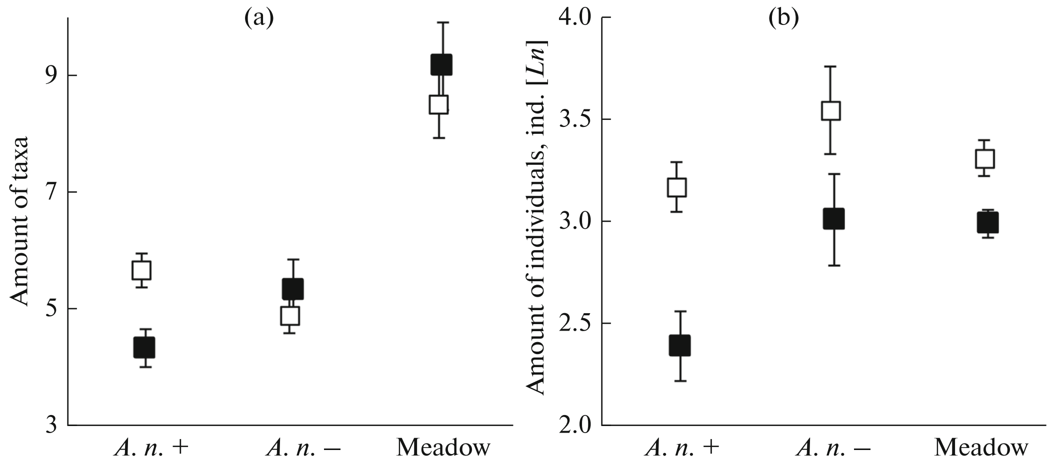

Fig. 1. Amount of taxa (a) and seedlings (b) in the variants of urban monospecific stands of *A. negundo* (*A.n.*+), urban areas without *A. negundo* (*A.n.*–), and countryside meadows in tour I (□) and tour II (■) ($m \pm SE$).

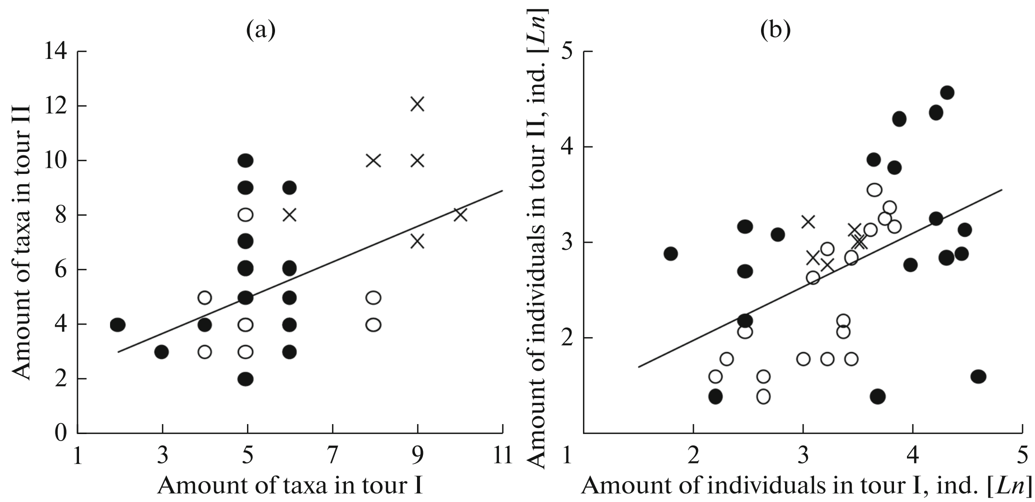

Fig. 2. Correlation between the amount of taxa (a) and seedlings (b) in tours I and II in the variants of urban monospecific stands of *A. negundo* (●), urban areas without *A. negundo* (○), and countryside meadows (×) ($m \pm SE$).

tours I and II was $r = 0.46$ ($p = 0.0021$) (Fig. 2a). The correlation coefficient between the logarithmic estimates of abundance in containers in tours I and II was $r = 0.47$ ($p = 0.0015$) (Fig. 2b). The presence of such dependences indicates that the mean values of abundance and diversity in tour II was related with the values in tour I.

Total amount of taxa in the variants. The analysis of cumulative curves of taxonomic diversity depending on the number of samples showed that the diversity was the highest in the countryside meadow area in both tours (Fig. 3). We found 17 taxa in 6 containers in tour I and 24 taxa in tour II; extrapolated values for $n = 18$ are even higher (24 and 31 species, respectively). Differences between variants *A.n.*+ and *A.n.*– are insignificant and unstable. The taxonomic diversity in tour I was higher on the soils from *A. negundo* thickets, and in tour II, it was higher in the control soils.

DISCUSSION

The diversity of plants in living vegetation cover in plant communities with *A. negundo* has been studied a number of times (Ryabinina and Nikitina, 2009; Emel'yanov and Frolova, 2011; Kostina et al., 2015; Gusev, 2016; Gusev et al., 2017). It was found that the invasion of *A. negundo* leads to the reduction of diversity of other plant species. The negative influence of this invasive species on herbaceous plants is especially obvious in communities that we studied: the number of herbaceous species in *A.n.*+ variants was 2–8, while in *A.n.*– it was 10–15 (Table 1). The abundance of herbaceous plants in the monospecific stands of

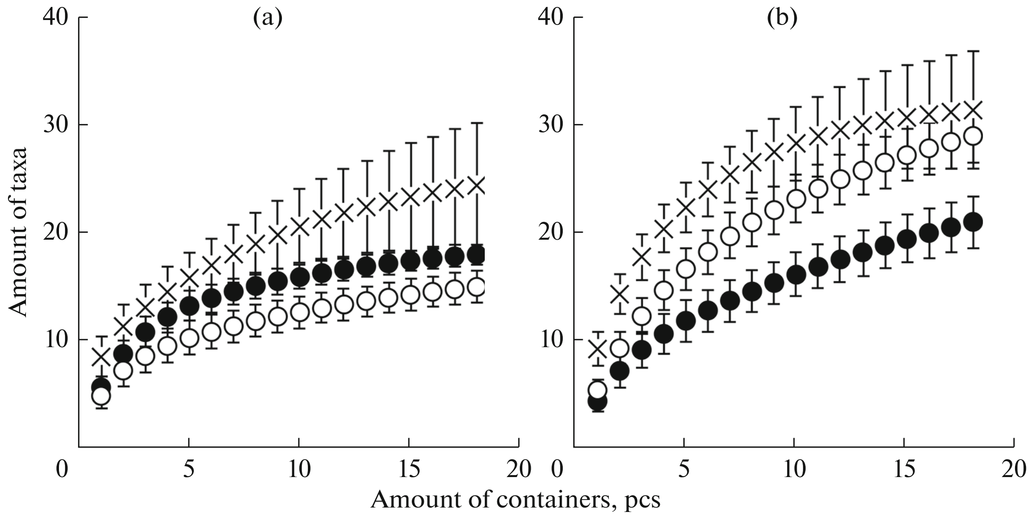

Fig. 3. Cumulative amount of taxa of seedlings in tours I (a) and II (b) in the variants of urban monospecific stands of *A. negundo* (●; interpolated estimates), urban areas without *A. negundo* (○; interpolated estimates), and countryside meadows (×; extrapolated estimates after $n = 6$) ($\pm SD$).

A. negundo was reduced significantly (by 5–10 times or even more).

As compared with such effect on the plant species of vegetation cover under the canopy of *A. negundo*, its effect on the abundance and diversity of seedlings of the seed bank is less significant. The taxonomic diversity in one container or in one variant of experiment was unaffected by the presence of *A. negundo.* The mean amount of taxa in one container (Fig. 1a) or in the whole variant (Fig. 3) did not differ between the *A.n.*+ and *A.n.*– variants; differences in abundance were significant only between all urban variants (*A.n.*+ and *A.n.*–) and countryside meadow areas. The effect of *A. negundo* on the abundance of seedlings is more significant than that on the parameters of taxonomic diversity. The urban habitats differ significantly in this parameter: the abundance of seedlings in the thickets of *A. negundo* is lower by 1.5–2.5 times than in ruderal habitats without *A. negundo* (Fig. 1b).

Such a small effect of *A. negundo* on the abundance and diversity of seedlings corresponds with other published data on the effect of invasive species on soil seed banks. Invasive tree species (trees and shrubs) are known to affect seed banks to a lower extent as compared with foreign herbaceous species (Gioria and Osborne, 2014). Our conclusion on lower sensitivity of soil seed banks to the influence of invasive plant species as compared with vegetating species is also consistent with published data (Gooden and French, 2014).

Owing to the methodological difficulties, we did not estimate or discuss the taxonomic structure of seedling communities. We did not isolate soil in our containers from possible influence of dispersal units contained in the air, as is usually done when investigating the composition of seedlings (*Metody*..., 2002). Therefore, we cannot exclude the effect of some seeds that could have invaded soil samples after we collected them, sieved, and placed them into the containers. However, our experiment was carried out in a greenhouse isolated from the environment. This provides lower probability of random seed invasion by the air flow. In general, additional contamination can result in changes in the estimates of abundance and diversity of the seed banks. On the other hand, we suppose that such changes could not be significant, since intense contamination in the containers would not provide such correlation in the parameters of abundance and diversity between the tours.

CONCLUSIONS

This study provides the first analysis of the parameters of the soil seed bank in communities transformed by *Acer negundo*. It was found that the taxonomic diversity of seedlings from the seed bank in ruderal urban areas is close regardless of the presence of invasive *A. negundo*. The influence of *A. negundo* on the amount of viable seeds in soil is the most significant. The general influence of this species on the soil seed bank in Yekaterinburg is not very significant, even though this is an absolutely dominant species in the tree layer, which causes the reduction of diversity of vegetating plant species.

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