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Influence of anthropogenic disturbance on the number and composition of plant species in sparsely populated areas: a case study of Udomlya district (Tver region, European Russia)

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ABSTRACT

Question: What is the relationship between the number of native and non-native plant species in sparsely populated territories? How does anthropogenic disturbance influence plant species composition and diversity? Do non-native plants increase the homogeneity of the flora?

Data: The flora of Udomlya district (Tver region, central part of European Russia) mapped into 96 squares, each 5 km × 5 km.

Method: We searched for correlations (corrected for spatial autocorrelation) between the number of native and non-native plant species, and indicators of anthropogenic habitat transformation. We also used cluster analysis (Jacquard's similarity coefficient, Ward's method of agglomeration) to determine whether the squares cluster into floristic groups, and if so, into how many clusters. Finally, we determined indicator species for each group based on the IndVal index.

Conclusions: Both the number of native and non-native plant species increases with anthropogenic or natural disturbances in association with high landscape heterogeneity. Non-native plants decrease the homogeneity of the flora. We found two floristic groups of squares. A square belonged to a group depending mainly on that group's degree of anthropogenic transformation.

Keywords: plant species diversity, plant richness, invasive species, anthropogenic disturbance, non-native species, floristic composition, Udomlya district, Tver region, Russia.

INTRODUCTION

Increased anthropogenic disturbance has degraded biodiversity [usually expressed as the number of species (Petřík *et al.*, 2010)] in the environment (Vogtländer *et al.*, 2004). Ecosystems with anthropogenic disturbance contain many non-native species (Godefroid, 2001; McKinney, 2002).

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However, it is still unclear how the diversity of non-native species influences the diversity of native species.

Traditionally, the introduction of non-native plants and their establishment was thought to cause a decline in the number of native species (Williamson, 1999). However, research has revealed positive relationships between the number of native and non-native plant species in some quite densely populated areas of Western Europe and America (e.g. Rosenzweig, 2001; McKinney, 2002; Sax, 2002; Deutschewitz *et al.*, 2003; Kühn and Klotz, 2003; Kühn *et al.*, 2003). The explanation given for this positive relationship is that the two measures of diversity are explained by the same mechanism – that is, the number of both native and non-native species is enhanced by greater variability of environmental factors or landscape diversity (Deutschewitz *et al.*, 2003; Kühn and Klotz, 2003; Kühn *et al.*, 2003). Moreover, historical land use and settlement also followed the distribution patterns of natural resources (Deutschewitz *et al.*, 2003). That is especially so in rural areas where links between anthropogenic disturbance and the number of species should thus be even more indirect. It would be informative to know whether these positive relationships between the number of native and non-native species can be generalized across various environments, including sparsely populated areas (Sax, 2002; Kühn *et al.*, 2004).

Biological invaders were supposed to homogenize floras, making them less variable owing to replacement of various native species by a few successful invaders (McKinney and Lockwood, 1999). However, this idea is not supported by an analysis of neophytes in the German flora (Kühn *et al.*, 2003).

Last but not least, the known number of species depends also on recording effort, such as the number of researchers involved (Petřík *et al.*, 2010), their qualifications and recording methods (Rich and Woodruff, 1992; Rich, 1998), and the amount of time devoted to the task. These variables are seldom measured and reported, causing uncertain sampling bias (Kühn and Klotz, 2003; Kühn *et al.*, 2004), leading to the idea that 'the distribution of plants reflects the distribution of botanists' (Rich and Woodruff, 1992).

There are many detailed floristic studies based on grid mapping in densely populated areas of Western and Central Europe (Petřík *et al.*, 2010) but few have been conducted in the sparsely populated areas of Eastern Europe. In Russia, grid mapping has been used for floristic studies in one region only: the region of Vladimir, in the central part of European Russia (Seregin, 2014). However, grid mapping was also used in European Russia for the orchids of the northwestern regions, for the aquatic plants of the Tula region (reviewed in Seregin, 2014), and for several archipelagos in the Gulf of Finland [Baltic Sea (Glazkova, 2001)]. Those studies are largely descriptive in nature, seeking to obtain more accurate estimates of species' abundances and distributions. With so few data, we are unclear about the generality of the patterns revealed in Western and Central Europe. Equally, limited information is available for territories with low anthropogenic disturbance.

Faced with this paucity of data, we conducted grid mapping of the flora in the district of Udomlya, a sparsely populated area with uniform natural and land use history in the middle of European Russia (see 'Study area' below). We wished to test the following hypotheses:

1. The number of native plant species is positively correlated with the number of non-natives.
2. Anthropogenic disturbance increases both native and non-native plant diversity.
3. Non-native plant species decrease the homogeneity of the flora.

MATERIALS AND METHODS

Study area

Udomlya district, in the northernmost part of the Tver region (European Russia), has an area of approximately 2500 km^2 (57°41′18″to 58°13′20″N, 34°25′10″ to 35°20′00″E). The district is situated in the Valdai Hills, which is the watershed between the Baltic and Caspian seas (East European plain). It has low topographical ruggedness, ranging from 130–140 m in the lowlands in the eastern, southern, and northwestern parts of the district, to 180–220 m in the uplands in the central and northeastern parts. The district forms part of the southern taiga vegetation zone. Mean temperature of the coldest month (January) is −8.8°C, while that of the warmest month (July) is 16.9°C. Mean rainfall is around 700 mm. Udomlya district has numerous lakes and rivers. The three main rivers are the Msta in the west, the Sjezha in the central zone, and the Volchna in the south (Fig. 1). For a description of the study area, see Vinogradov *et al.* (1999).

Udomlya district is characterized by well-preserved natural vegetation and low human population density (*c.* 18 individuals/km^2). Most of the population (75%; *c.* 8880 individuals) is concentrated in the town of Udomlya, towards the centre of the district (Fig. 1). Mean density of the rural population is 4.6 individuals/km^2. Forests, which cover about

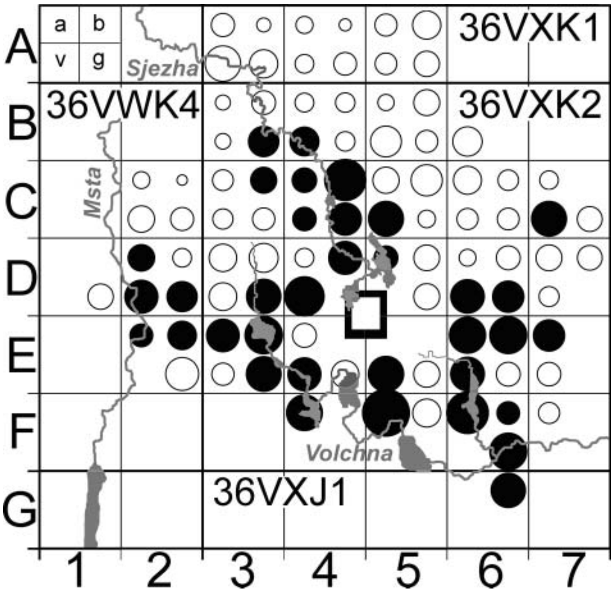

Fig. 1. Squares of Udomlya district. Each 5 km × 5 km square is represented by a circle whose area is proportional to the number of species it contains. The shade of the circle identifies the floristic group of the square (black: *Artemisia* group; white: *Pyrola* group; see text for more details). Thin lines correspond to borders between tetrads of the studied squares; their labels (letters and numbers) are given on the axes. Labelling of each square within a tetrad is shown by the example in the upper left tetrad. Thicker lines mark the grid-cells of *Flora Europaea*, their labels indicated inside (e.g. 36VWK4). The very bold square at the centre indicates the only town in the district – Udomlya. Main rivers are shown in grey. Squares not included in the study are omitted.

60% of the district's area, are dominated by Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) Karst.), silver birch (*Betula pendula* Roth), downy birch (*Betula pubescens* Ehrh.), and European aspen (*Populus tremula* L). Lowland areas (*c.* 6% of the district) are in the most part covered with peat and back bogs. The rest of the district comprises fields, settlements, and roads. Beginning in the tenth century, the practice of slash-and-burn agriculture saw most forests replaced with fields. This process became most intensive with the advent of modern agriculture during the transition from the nineteenth to the twentieth century. The intensity of agriculture had decreased substantially by the end of the twentieth century and most fields today have been abandoned.

Plant data

Udomlya district represents almost the whole of one grid-cell (i.e. $36VXK_2$) of *Atlas Florae Europaeae* (https:www.luomus.fi/en/atlas-florae-europaeae-afe-distribution-vascular-plants-europe). It also includes small fragments of the three adjacent cells (Fig. 1). We divided the district into squares, each 5 km × 5 km. In 2007–2015, we obtained presence/absence data for the species of 96 squares representing almost the entire region. (The data matrix can be downloaded at www.evolutionary-ecology.com/data/3031Appendix.pdf or ashipunov.info/shipunov/open/flr_sq.csv.) We left a few marginal squares unexplored because less than 50% of the territory of each was located in Udomlya district (cf. Nogués-Bravo and Araújo, 2006). We also did not include the five central squares that mainly belong to the town of Udomlya (Fig. 1), part of which could not be accessed for floristic studies due to the nuclear power plant situated there. Another reason to exclude these urban squares from our analysis is a well-known peculiarity of urban flora (Godefroid and Koedam, 2003; Vähä-Piikkiö *et al.*, 2004), which would have introduced bias in our results.

We minimized sampling bias by working in a single team of well-trained botanists according to a single sampling protocol. Our field research group always consisted of two senior botanists (L. Abramova and P. Volkova), accompanied by one to three students. We visited all types of habitats in a square, planning our route beforehand using satellite and topographic data. All investigations were done in the same season (the last week of June and the first week of July). As the training of researchers is essential to minimize sampling bias (Rich and Woodruff, 1992), we assessed 16 squares during a preliminary study in 2006, the data for which were not included in the analyses. We also tried to standardize recording coverage, spending one working day (6–10 hours) in each square. But the length of the route varied considerably depending on the landscape (3031Appendix, Fig. A1). Finally, we addressed the fact that plant species of several genera are poorly distinguished in the field (*Alchemilla*, *Bidens*, *Euphrasia*, *Hieracium* excluding *H. umbellatum* aggr., *Pilosella* spp. excluding *P. officinarrum* aggr.). To minimize identification bias (*sensu* Rich and Woodruff, 1992), we did not try to identify individuals of these genera to the species level. Instead, we collected and delivered them to taxonomic experts for identification. Then, we deposited them in the herbarium of Moscow State University (MW). Thus we endeavoured to avoid the usual causes of sampling bias (Rich and Woodruff, 1992; Rich, 1998; Kühn and Klotz, 2003; Kühn *et al.*, 2004; Diekmann *et al.*, 2008; Petřík *et al.*, 2010).

The floristic survey took into account both native and non-native (sometimes termed 'alien') species. The latter mainly included neophytes (reliable data on the species history in the Tver region are available from the end of the eighteenth century), but also some archaeophytes, whose abundances have changed substantially since the beginning of the twentieth century (Notov, 2009). Based on the degree of naturalization in the Tver region (Notov 2009), non-native species were classified as:

- *casuals* or ephemerophytes: these lack self-reproducing populations and persist only during their individual development;
- *naturalized* or colonophytes: these persist at the place of introduction for some time due to vegetative or seed reproduction, but do not spread;
- *invasives* or epecophytes: these spread in habitats that are disturbed by humans;
- *transformers* or agriophytes: these become part of natural communities.

Protected species were listed according to the *Red Book of the Tver Region* (2002) and the *Red Book of the Russian Federation* (2008).

Data analysis

We used human population per square, obtained from Vinogradov *et al.* (1999), as an effective indicator of modern anthropogenic pressure (Moraczewski and Sudnik-Wyjcikowska, 2007). We analysed the log-transformed values of population because the value of this variable spans four orders of magnitude (0–1075 individuals per square). As an indicator of landscape transformation by humans and of the intensity of agriculture in the past, we calculated the area of fields at the end of the twentieth century from topographic maps (scale 1 : 100 000) (Vinogradov *et al.*, 1999).

We classified the squares based on their floristic composition using cluster analysis with Jacquard's similarity coefficient, which reflects the relative number of species that are common to a given pair of squares. We considered that negative coincidences (absence of the species in both squares) should not be taken into account owing to the possibility of missing species by chance (Rich, 1998). We used Ward's method of agglomeration because it results in compact clusters. Typical species for the given group of squares (cluster) were revealed with the IndVal index. This index is based on a combination of specificity and fidelity of the species to that group of squares. The value is maximal when a species occurs in all squares of a group and is absent from all squares assigned to other groups (Dufrêne and Legendre, 1997). To determine any correlation between similarity matrices, calculated separately for native and non-native species, we performed a Mantel test (Mantel, 1967).

It is likely that some species in a square were missed owing to the finite nature of our sampling process. To estimate the number of species missed, we used species accumulation curves (Gotelli and Colwell, 2001). This method reveals the growing number of species encountered as a result of increasing the number of squares studied. We estimated the statistical significance of associations between quantitative variables with Spearman correlation tests. We estimated the significance of between-group differences with Wilcoxon tests.

Since plant distribution patterns are likely to be influenced by spatial autocorrelation, neighbouring squares usually have a higher probability of being similar to each other than squares further apart. Testing significance without taking spatial autocorrelation into account could result in an overestimation of the degrees of freedom (Deutschewitz *et al.*, 2003). However, in our case, results of a modified *t*-test of Dutilleul (1993), which accounts for spatial autocorrelation, and a standard Pearson's correlation test after normalizing skewed data were very similar (not shown). Such similar results indicate the absence of spatial autocorrelation in our data. Thus we used standard non-parametric Spearman correlation tests as the distribution of the values still deviated from normal, even after their normalization.

All computations and graphics were made using the R statistical environment (R Development Core Team, 2013).

RESULTS

Native and non-native species

During the survey, we recorded 775 taxa (including hybrids), of which 149 were non-natives. The numbers of native and non-native species in a square were strongly positively correlated (Pearson's correlation test: $P = 2.2 \times 10^{-16}$, $r = 0.8$; Fig. 2).

There was a positive relationship between the dissimilarity coefficients of native and non-native species (Mantel test, 999 permutations: $P < 0.001$, $r = 0.43$). Dissimilarity coefficients were significantly greater for non-natives than for natives (Wilcoxon test: $P = 2.2 \times 10^{-16}$; mean ± SD: 0.7 ± 0.11 and 0.4 ± 0.06 respectively).

The accumulation curve for native species had a steeper initial slope and began to reach an asymptote, whereas the accumulation curve for non-native species was still climbing after all squares had been included (3031Appendix, Fig. A3). The curvature of the accumulation curve (expressed as the slope of the curve in the logarithmic space) was higher for native plants than for non-natives (mean ± SD: 0.13 ± 0.004 and 0.37 ± 0.004 respectively).

The number of non-natives in a square was positively correlated with population and area of fields (Spearman's correlation test: $P = 1.4 \times 10^{-14}$, $R = 0.69$ and $P = 6.4 \times 10^{-7}$, $R = 0.48$ respectively). Area of fields explained a higher proportion of the variance in the number of invasive species ($R = 0.5$; $n = 53$ species) than in the number of casual or

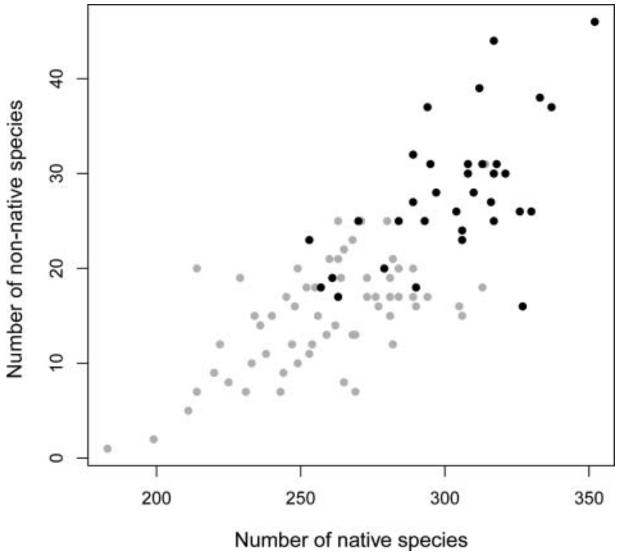

Fig. 2. The positive relationship between the numbers of native and of non-native species per square for all squares from the two floristic groups (the *Artemisia* group – black dots; and the *Pyrola* group – grey dots; see text for details).

naturalized non-natives ($R = 0.3$; $n = 55$ or 39 species respectively). There was no such difference between groups of non-native plants in respect of population influence. Transformers were not analysed separately, as they were represented by only two species (*Amelanchier spicata* (Lam.) K. Koch and *Elodea canadensis* Michx.). In general, non-native species were less frequent than native species (Wilcoxon test: $P = 2.2 \times 10^{-16}$; mean ± SD: 20 ± 13.1 and 44 ± 34.8 grid-cells respectively).

Grouping of grid squares

Cluster analysis revealed two main groups of squares based on their floristic composition (Fig. 3). The ten most indicative species for each group are presented in Table 1. We named the groups after the species with the highest IndVal value (*Pyrola rotundifolia*, hereafter the *Pyrola* group, 61 squares; and *Artemisia campestris*, hereafter the *Artemisia* group, 35 squares). There was significant overlap. Many species that were typical for the *Pyrola* group were not unusual for the *Artemisia* group. Thus we selected as characteristic for the *Pyrola* group those species with the highest IndVal value for this group and the lowest IndVal value for the *Artemisia* group. Number of species per square was significantly lower in the *Pyrola* group than in the *Artemisia* group ($P = 6.8 \times 10^{-11}$; 95% confidence interval [CI]: 271–287 and 326–347 species respectively; Fig. 3).

Table 1. List of the ten most indicative plant species, based on their IndVal value (cf. Dufrêne and Legendre, 1997), for each of the two main groups of squares

		IndVal	
Group	Species	*Pyrola* group	*Artemisia group*
Pyrola	*Pyrola rotundifolia*	0.52	0.38
	Chrysosplenium alternifolium	0.52	0.42
	Carex disperma	0.51	0.12
	Vaccinium uliginosum	0.51	0.14
	Orthilia secunda	0.50	0.37
	Pulmonaria obscura	0.48	0.23
	Geranium sylvaticum	0.47	0.25
	Ledum palustre	0.41	0.22
	Rubus chamaemorus	0.41	0.22
	Linnaea borealis	0.41	0.23
Artemisia	*Artemisia campestris*	0.04	0.70
	Campanula persicifolia	0.12	0.66
	Silene alba	0.12	0.66
	Quercus robur	0.16	0.65
	Viola arvensis	0.19	0.65
	Chamomilla suaveolens	0.21	0.64
	Bromopsis inermis	0.08	0.63
	Thlaspi arvense	0.10	0.63
	Medicago lupulina	0.24	0.62
	Chelidonium majus	0.08	0.61

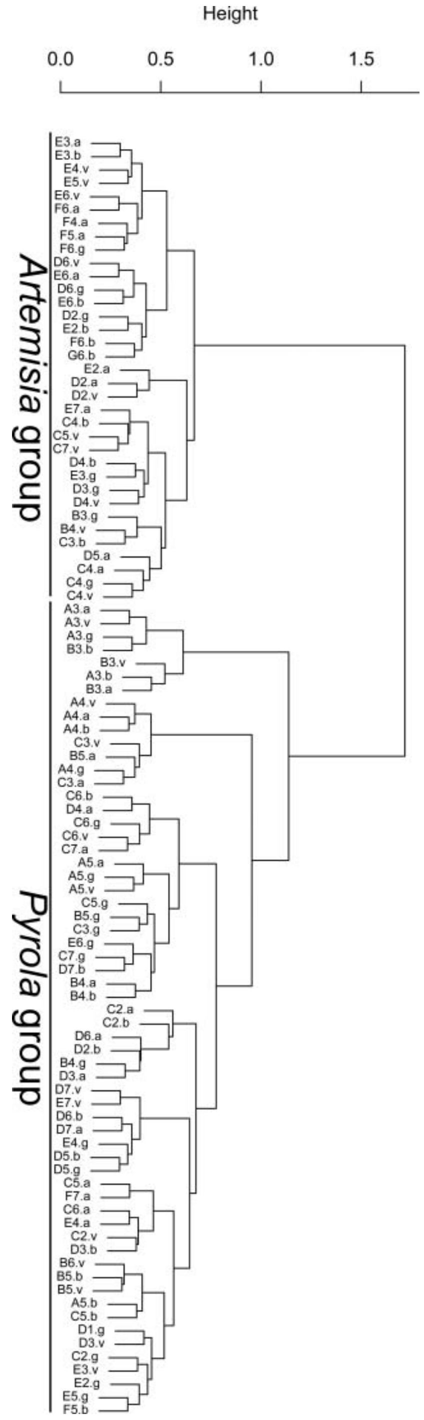

Fig. 3. Classification of the squares according to their floristic composition (Jacquard's similarity coefficient; Ward's method of agglomeration). The two main groups of squares are the *Artemisia* group and the *Pyrola* group (see text for more details).

Squares of the *Pyrola* group were characterized by a significantly smaller area of fields (Wilcoxon test: $P = 0.0001$; 95% CI: 14–21% and 29–45% respectively) and smaller human population ($P = 1.1 \times 10^{-9}$; 95% CI: 15–42 and 136–313 individuals respectively) compared with those of the *Artemisia* group, although area of fields and population varied widely in both groups (3031Appendix, Fig. A2). The combination of these characters was important. For example, because no one lived in square D5-b, it belonged to the *Pyrola* group in spite of its high area devoted to fields (*c*. 70%) (3031Appendix, Fig. A2).

Protected species

During the survey, we encountered 40 species that are protected in the region – that is, listed in the *Red Book of the Tver Region* (2002). Four of these species are listed in the *Red Book of the Russian Federation* (2008). Almost half of the 40 species were not rare in Udomlya district; we found them in more than 10% of the studied squares (Table 2). There was a weak negative correlation (Spearman's correlation test: $P < 0.002$, $R \approx -0.3$) between the number of protected plant species in a square and each of number of non-native species, field area, and human population. The number of protected species was slightly higher in squares of the *Pyrola* group than those of the *Artemisia* group (Wilcoxon test: $P = 0.01$; mean ± SD: 6.8 ± 2.80 and 5.34 ± 2.35 respectively).

DISCUSSION

Our results show clearly that, in plants, the number of native and non-native species is increased by anthropogenic or natural disturbances just as it is by higher landscape heterogeneity. In addition, as we shall discuss below, non-native plants actually decrease the homogeneity of the flora. Finally, the existence of the two main floristic groups of squares seems to reflect differences in transformation of the district by people.

Sampling bias is typical of most floral mapping projects because the data are gathered by many volunteers and with unknown sampling quality (Rich and Woodruff, 1992; Rich, 1998; Kühn and Klotz, 2003; Kühn *et al.*, 2004; Moraczewski and Sudnik-Wyjcikowska, 2007; Diekmann *et al.*, 2008; Petřík *et al.*, 2010). Our results, however, are as free from sampling bias as we could make them. And, as we discuss below, our findings are in line with the results of floral mapping obtained in large-scale, 'volunteer' projects after correction for sampling intensity (e.g. Kühn and Klotz, 2003; Kühn *et al.*, 2004; Diekmann *et al.*, 2008). Thus, even the non-uniform efforts of numerous volunteer botanists may yield quality information on floristic diversity.

We revealed a highly significant correlation between the similarity matrices for native and non-native species. This was also shown in Germany and it means that non-native species follow the biogeographic pattern that natives do (Deutschewitz *et al.*, 2003; Kühn *et al.*, 2003). This finding is supported by a strong positive relationship between the numbers of native and non-native species in a square, as was shown previously (Kühn *et al.*, 2003 and references therein). We already knew that the number of native and non-native plant species is increased by anthropogenic or natural disturbances in association with high landscape heterogeneity (Deutschewitz *et al.*, 2003; Vähä-Piikkiö *et al.*, 2004; Petřík and Wild, 2006; Diekmann *et al.*, 2008). A caveat: obtaining a correlation between native and non-native species does not mean there must be a cause and effect relationship; the underlying mechanisms may be different but correlated, confounding cause and effect (Kühn *et al.*, 2004).

Table 2. Protected plant species of the Tver region and their abundance in Udomlya district

Species	Status[a]	Abundance[b]
Arctostaphylos uva-ursi	2	3
Baeothryon alpinum	2	3
Betula humilis	2	2
Betula nana	2	1
Botrychium virginianum	3	1
Carex disperma	3	59
Carex magellanica	2	19
Corallorhiza trifida	2	18
Cypripedium calceolus	2*	4
Dactylorhiza baltica	4*	4
Dactylorhiza traunsteineri	4*	1
Delphinium elatum	3	1
Drosera anglica	2	9
Empetrum nigrum	2	22
Epipactis palustris	2	16
Epipogium aphyllum	1*	3
Equisetum variegatum	3	2
Galium triflorum	2	39
Gymnadenia conopsea	2	49
Hepatica nobilis	2	82
Huperzia selago	2	17
Jovibarba sobolifera	2	1
Lactuca sibirica	3	5
Listera cordata	3	10
Malaxis monophyllos	2	13
Moneses uniflora	2	23
Neottia nidus-avis	2	18
Nuphar pumila	2	4
Oxycoccus microcarpus	2	4
Platanthera chlorantha	4	50
Pulsatilla patens	2	1
Rhamnus cathartica	3	1
Rhynchospora alba	2	7
Rubus chamaemorus	2	61
Salix myrtilloides	2	20
Salix phylicifolia	3	1
Sparganium angustifolium	2	1
Utricularia intermedia	2	13
Utricularia minor	2	7
Viola uliginosa	3	2

[a] Status of species in the *Red Book of the Tver Region* (2002): 1, endangered; 2, vulnerable; 3, rare; 4, data deficient. Asterisks mark species of national concern included in the *Red Book of the Russian Federation* (2008). [b] Number of squares in which the species was found ($N = 96$).

Like Kühn *et al.* (2003), our results do not support the idea that non-native plants lead to homogenization of the flora (see McKinney and Lockwood, 1999). Rather, we found that the non-native components of the dissimilarity coefficients were significantly higher than the native ones. One explanation for this is stochastic establishment of non-natives, and the patchy distribution of disturbed areas in the sparsely populated district of Udomlya. Spatial heterogeneity of the non-native species distribution means that more sampling effort is needed to reveal the composition of non-native species compared with native ones; this is also suggested by the shape of the species accumulation curves (3031Appendix, Fig. A3) (cf. Thomspson and Withers, 2003). Both the steeper initial slope of the accumulation curve for native species, and its rapid approach to an asymptote, support our conclusion that natives are more abundant than non-natives. Lower average abundance of non-native plants could be explained not only by the limited area of disturbed habitats but also by an historical component – that is, insufficient time may have elapsed since their introduction. Indeed, many non-native species are still expanding their ranges in Europe (Williamson *et al.*, 2009). This historical component is especially applicable to the district of Udomlya, as it has a relatively low density of transportation routes and of settlements that could act as centres of plant introduction (Notov, 2009).

The existence of the two main floristic groups of squares is explained mainly by uneven anthropogenic transformation of the district. More disturbed squares (the *Artemisia* group) include rivers with roads and settlements along them. However, we were unable to perform statistical analysis of the influence of roads and rivers on the floristic composition because the available maps have low thematic resolution. For example, digitizing the road network in a square from existing topographic maps does not distinguish between different road uses (cf. Petřík and Wild, 2006 and references therein).

More intensive anthropogenic pressure on a square, both now and in the past, is indicated by a significantly higher population and area covered by fields. This finding is supported by the occurrence of characteristic species for the *Artemisia* group, species that inhabit open, usually disturbed habitats (mainly fields and roadsides). The only exception seems to be *Quercus robur*, but oak forests are absent in Udomlya district and this species grows in the understorey or is cultivated (Vinogradov *et al.*, 1999). The higher anthropogenic pressure in the *Artemisia* group of squares is associated with a higher number of non-native species. Naturally, habitats transformed by humans promote the expansion of non-native species (Godefroid, 2001; McKinney, 2002; Deutschewitz *et al.*, 2003), as is illustrated by the strong positive correlation between non-natives and area covered by fields. However, the number of species in the *Artemisia* group is also increased owing to the higher number of native species. First, as was mentioned above, moderate anthropogenic pressure generates greater diversity of habitats, which can be inhabited not only by non-native but also by native species (Deutschewitz *et al.*, 2003; Vähä-Piikkiö *et al.*, 2004; Petřík and Wild, 2006; Diekmann *et al.*, 2008). Second, rivers with formed valleys typical of squares from the *Artemisia* group also add to habitat diversity (Burkart, 2001; Deutschewitz *et al.*, 2003). Finally, historical land use and settlements followed the distribution patterns of natural variables (Deutschewitz *et al.*, 2003; Otýpková *et al.*, 2011). Settlements were usually established in geologically diverse areas, including large rivers (Kühn *et al.*, 2004). This means that the influence of anthropogenic pressure and landscape properties on the number of species is complex; for example, German cities are still rich in native species despite urbanization (Kühn *et al.*, 2004).

More effort will be needed to determine the entire flora of squares in the *Artemisia* group. These squares have higher habitat diversity and more numerous, stochastically distributed non-native species. This problem is illustrated by a pattern involving the length of the research route. A pattern that we observed in the *Artemisia* group but not in the *Pyrola* group is that the longer the research route in a square, the more species we listed (3031Appendix, Fig. A1).

Squares of the *Pyrola* group are characterized by species that are typical of various types of forest (Table 1). We expected this because squares of the *Pyrola* group have lower anthropogenic pressure (as indicated by their human population, field area, and number of non-native species) and, as a result, more forests (as discussed above). That is why forest plant species are typical in these squares.

The relatively high number of protected species (i.e. those included in the *Red Book of the Tver Region*, 2002) observed in squares of the *Pyrola* group is most likely not a result of lower competition with non-native species (McKinney and Lockwood, 1999), as the two sets of species have different habitat preferences (forest and anthropogenic respectively). Almost all protected species in our case are typical of forests (Table 2), so their number is higher in squares of the *Pyrola* group where more area is forested. The weak negative correlation between the number of non-native and the number of protected species probably does not reflect direct negative interactions between these species groups and is mediated instead by differences in anthropogenic pressure. Moreover, many species that are included in the *Red Book of the Tver Region* (2002) are quite common in Udomlya district (Table 2) and not endangered there.

We believe there are two reasons for the abundance of protected species in Udomlya district. First, its landscapes have low average anthropogenic disturbance and are quite often pristine compared with those in the Tver region as a whole. Second, grid mapping is very effective at discovering protected species. Seregin (2014) found similar floristic differentiation of grid-cells with relatively low and high anthropogenic transformation in Meschera National Park (Russia, Vladimir region), while Petřík and Wild (2006) found the same in Northern Bohemia (Czech Republic). Thus, patterns of floristic differentiation seem to be similar for sparsely populated areas all across Europe.

Overall, our results suggest that anthropogenic pressure in sparsely populated areas does not lead to decreases in the number of native or non-native plant species, which is at odds with the views of some others (e.g. Williamson, 1999; Vogtländer *et al.*, 2004). However, our results find support in recent studies of the German flora (Deutschewitz *et al.*, 2003; Kühn and Klotz, 2003; Kühn *et al.*, 2003), and are in line with the general prediction made by Rosenzweig (2001). Certainly our results do not mean that people and their works enhance biodiversity. First, as discussed above, the positive relationship between intensity of anthropogenic pressure and the number of species is not direct but occurs because of their dependence on the variability of the same environmental factors. Also, anthropogenic pressure usually leads to habitat fragmentation, which may result in smaller and more vulnerable populations (Endels *et al.*, 2002). We did not address this issue here because, as is typical for grid mapping, we did not assess the population status of the plants. Nevertheless, a lower average number of protected species in more disturbed squares, which have less forested area, does suggest a possible negative effect of habitat fragmentation. Godefroid and Koedam (2003) found a similar positive relationship between the number of species and woodlot area in the Brussels region. Finally, the number of species is not the best indicator of environmental impact, although it is the most straightforward and most commonly applied. Its main problems are that it assumes all species have equal value (both invasive and endangered) and neglects the importance of unique but species-poor ecosystems (Vogtländer *et al.*, 2004). Other, possibly more useful indicators of environmental impact such as the rare ecosystems approach (Vogtländer *et al.*, 2004) and the estimation of species density (Rosenzweig *et al.*, 2011) have been proposed, but both the scientific community and policy-makers are yet to embrace them.

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