

Twin plots – appropriate method to assess the impact of alien tree on understory?

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Kľúčne besede: nasadi robinije, spremembe vrstne sestave, pari popisov, *Chelidonio majoris-Robinion*, *Fraxino-Quercion roboris*, različnosť, raznolikost.

Abstract

Replacing native forests by alien tree plantations can lead to changes in the species composition of the understory. However, differences in the understory species spectrum can also be a part of the natural variability of forest stands. We have tested the suitability of the twin plots method for an evaluation of the impact of alien trees on the species composition of the understory. This research was conducted on an alluvial plain (SW Slovakia) that was originally covered by a hardwood floodplain forest. The study was based on 7 twin plots of black locust (*Robinia pseudoacacia*) and native forest plots, with a maximum distance of 100 meters between the members of the twins. The dissimilarity of the plots within the black locust forest was significantly lower than the dissimilarity between the twin plots. In addition, the dissimilarity of the plots within the hardwood floodplain forest was also significantly lower than the dissimilarity between the twin plots. Under the same environmental conditions, the higher dissimilarity of the twin plots was caused by major edificators and their impact on the understory vegetation. The twin plots method proved to be a suitable tool for analyses of the impact of alien trees on understory vegetation.

Izveček

Nadomeščanje naravnih gozdov z nasadi tujerodnih vrst lahko vodi v spremembe vrstne sestave zeliščne plasti. Vendar so lahko razlike v vrstni sestavi zelišč tudi del naravne variabilnosti gozdnih sestojev. Testirali smo primernost metode dvojnih popisnih ploskev za ovrednotenje vpliva tujerodnih drevesnih vrst na vrstno sestavo zeliščne podrasti. Raziskavo smo izvedli na poplavni ravnini (JZ Slovaška), ki je bila prvotno poraščena z trdolesnimi poplavnimi gozdovi. Temeljila je na sedmih dvojnih popisnih ploskvah robinije (*Robinia pseudoacacia*) in popisnih ploskvah naravnega gozda. Razdalja med njimi je bila največ 100 metrov. Različnosť med ploskvami v sestojih robinije je bila statistično značilno manjša kot razlika med dvojnimi ploskvami. Tudi različnosť med ploskvami v trdolesnem poplavnem gozdu je bila značilno manjša kot med dvojnimi ploskvami. V enakih rastiščnih razmerah je bila večja raznolikost med dvojnimi ploskvami posledica glavne edifikatorske vrste in njenega vpliva na zeliščno plast vegetacije. Pokazali smo, da so dvojne ploskve uporabna metoda za analizo vpliva tujerodnih dreves na zeliščno podrast.

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Introduction

In many territories, alien tree species can establish themselves and behave invasively. They can disrupt the natural ecosystems (Richardson et al. 1989, Dickie et al. 2014) and can cause changes to the environment in many ways, consequently, modifying the species composition. As a result of the increments in global trade, urbanisation, land use intensification, as well as climate change, a growth in the number of established invasive species is expected (Dawson et al. 2017). For this study, we chose the black locust (*Robinia pseudoacacia* L.) as a model species, due to its high abundance in Central Europe (Vítková et al. 2016). The black locust species can add as much as 75 kg of nitrogen ha⁻¹ per year to the soil (Boring & Swank 1984), with a high probability that it facilitates the establishment of other invasive species (Peloquin & Hiebert 1999, Von Holle et al. 2006). The black locust spreads quickly, generatively and vegetatively, through its sprouts (Kowarik 1996, Jung et al. 2009). The above-mentioned properties of the black locust are important and effective tools for its reaching out to new habitats (Lee et al. 2004).

The impact of alien tree species on understory vegetation can be analysed in several ways. One method is by comparing natural and alien forest diversities by using large datasets from databases (Chytrý et al. 2005, Medvecká et al. 2012, Wagner et al. 2017). Another widely used approach is based on the comparison of alien and natural forest plots in an ecologically specified area (Riepšas & Straigytė 2008, Marozas et al. 2009, González-Muñoz et al. 2012, Woziwoda et al. 2014). The concept of paired “twin” plots represents another method, which has been used in both fertilisation experiments and forest production models in forestry research (Stape et al. 2004, Stape et al. 2006). In an invasive ecology, the twin plots method is based on the sampling of twin phytosociological relevés on both sides of the boundary between native and alien tree stands, at a close distance, with the aim of evaluating the impact of alien tree species on the understory vegetation. There have been several studies using twin plots in order to evaluate impact of alien species on species diversity (Dzwonko & Loster 1997, Hejda et al. 2009, Benesperi et al. 2012, González-Muñoz et al. 2012, Sitzia et al. 2012, Trentanovi et al. 2013). However none of these studies have verified whether the changes in floristic composition of the understory in alien tree stands, are only part of the natural variability of species composition in forest vegetation.

In analyses of forest twins (pairs of relevés), usually all of the differences in species composition between them can be explained by the influence of alien trees. This explanation is based on the assumption that if the environmental conditions of the twin plots are the same, we can

deduce that most of the differences are caused by the different dominating tree. However, phytocoenological relevés that have been previously recorded in the same type of native forest and under the same environmental conditions, with short distances, are not totally the same. They differ more or less in species composition and abundance, as a consequence of the natural variability of species composition, in every type of forest. The same is also true for alien tree dominated stands – they likewise display some natural variability.

In this study, we have tested the suitability of the methodology of twin plots, for the evaluation of the impact of alien tree species, on species diversity in the understory. If the similarity of relevés from the same type of forest is larger than the similarity between the twins of relevés (alien vs. native edificator tree (Sukachev 1964)), recorded under the same environmental conditions, then the twin plots method could be considered as being a suitable tool for the evaluation of impact of alien tree species on understory species composition.

Material and Methods

Study Area

The model's locality was chosen based upon LGIS - Geographic Information System for Foresters (<http://gis.nlcsk.org/lgis/>), which is available to users via the Internet, as a comprehensive information tool for science, research, economy, and public practice. LGIS contains information about forestry units, a list of planted tree species, the stands age, and so forth. It makes searching for the potential suitable twin plots more efficient. Sitzia et al. (2012) worked in their study with nearby sampling units. They claimed that the methodology would be best suited to areas where precise forest-type vegetation maps or clear historical photographs were available. LGIS, which was used in this study, was one of the best options for the selection of twin plots, because of its availability via Internet, as well as in its precise and actualised forest vegetation maps.

The model's locality was located in the Borská nížina Lowland, NNW of the Bratislava city (SW Slovakia; 16° 58' 54.4", 48° 17' 02.2"; 16° 59' 28.3", 48° 16' 56.7"; 154 m a.s.l.), on the alluvial plain of the Stupava creek (Figure 1).

According to the geobotanical map of Slovakia, the whole of the selected area has the potential to be covered by a hardwood floodplain forest (Michalko et al. 1987). The alluvial plain enables researchers to expect that all of the abiotic conditions on the studied plots are approximately the same. All of the relevés were taken from the forests on the alluvial plain along the Stupava creek

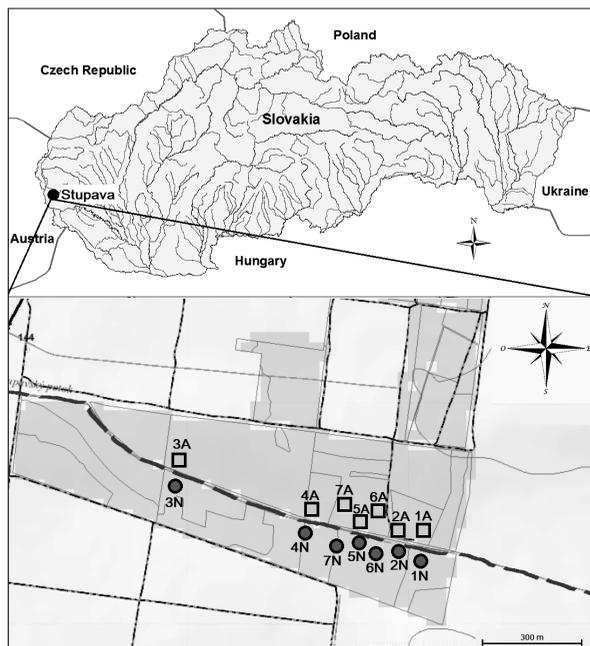


Figure 1: Location of the study area near to Stupava city (big black dot) in Bratislava district; distribution of twin relevés dominated by native oaks, elms and ashes (circles) and the stands dominated by alien black locust (squares); Stupava creek (dashed line) (<http://gis.nlcsk.org/lgis/>).

Slika 1: Lokacija preučevanega območja v bližini mesta Stupava (velika črna pika), v okrožju Bratislava; razporeditev dvojnih popisov z dominantnimi hrasti, bresti in jeseni (krožci) in sestoji z dominantno tujerodno robinijo (kvadratici); potok Stupava (črtkana črta) (<http://gis.nlcsk.org/lgis/>).

(54 ha), which belongs to the Territorial System of Ecological Stability (TSES) (http://www.stupava.sk/galeria/file/uzemny_plan-zmena2012/130502_zadanie_upnstupava_final_01.pdf). It contains elements of a regional importance, a regional biocorridor and a local biocentre (Ruzicka & Miklós 1982).

Recently, the Stupava creek has formed a natural border between the hardwood floodplain forest dominated by *Fraxinus angustifolia*, *Quercus robur*, *Ulmus laevis* and *Ulmus minor* on the left bank of the creek (25-75 years old) and the *Robinia pseudoacacia* forest on the right bank (15-45 years old). The tree composition has been minimally formed by black locust and native trees since 1955 (<https://www.staremapy.sk/?zoom=15&lat=48.28120807676698&lng=16.99058818810953&map=SR1952>). Therefore, the impact of tree species on understory vegetation has remained for at least for 60 years.

Data Collection

The dataset consisted of 14 relevés. All of the relevés were recorded by the methods of the Zürich-Montpellier school (Braun-Blanquet 1964) in the forest stands that

were dominated by black locust, classified within the alliance *Chelidonio majoris-Robinion* Hadač et Sofron ex Vítková in Chytrý 2013 (7 relevés) and stands dominated by native oaks, elms and ashes, classified within the alliance *Fraxino-Quercion roboris* Passarge 1968 (syn. *Ulmention* Oberd. 1953) (7 relevés).

The twin plots were always recorded on localities with the same altitude, aspect, slope, and soil type, on equal area, and at the same time. The only difference between the stands was the dominant tree. The sampling of the nearby twin plots (native vs. alien forest) ensured the uniformity of the abiotic factors (we can suppose that they were the same). In order to maximise the uniformity of the environmental conditions data, the samples were collected on a relatively small and homogeneous area. All of the relevés were collected on squared plots (20×20 meters) and contained a list of plant species, with their cover estimated by using the new Braun-Blanquet scale (Braun-Blanquet 1964, Westhoff & van der Maarel 1978), the date, and locality, as defined by the geographical coordinates that were measured by hand GPS Garmin Oregon 600t. The distance between the relevés of the twins was less than 100 metres. The upper limit in the study was the borders of forest area; the lower limit did not exceed a distance 30 meters. For a shorter distance than 30 meters, an edge effect appeared (Honnay et al 2002). For the nomenclature of higher vegetation units, we followed Jarolímek & Šibík (2008). The nomenclature of the vascular plants was in accordance with Marhold (1998).

Data Analyses

The data was stored in a TURBOVEG database (Hennekens & Schaminée 2001) and it was processed by using JUICE software (Tichý 2002). The species from the tree layer were omitted in order to focus on the differences in the herb layer. The different layers were merged and the species that occurred in only one relevé with cover “r” or “+” were deleted in order to avoid the influence of randomly occurring species. In order show the relations among all of the relevés, we used hierarchical cluster analyses in SYN-TAX 2000 (Podani 2001), with a beta-flexible algorithm ($\beta = -0.25$) and a Bray-Curtis dissimilarity coefficient. In order to test the significance of the differences in the species composition between the native and alien forests, we used PERMANOVA (R-software, Package Vegan). The distance measurements (dissimilarities) were calculated by SYN-TAX 2000 and were used for following data processing. Each relevé was calculated for “twin dissimilarity” – i.e. the dissimilarity between the current relevé and its related twin rel-

evé from the other forest type. The “control dissimilarity” was then calculated as a mean of the dissimilarities between the current relevé and all of the other relevés from the same forest type. “Control” and “Twin” dissimilarities were subsequently used, in order to compare the dissimilarities inside and between the groups. We checked for the normality of the distribution of the data in STATISTICA software (StatSoft 2001) when using the Kolmogorov-Smirnov test for normality. As the data had a non-normal distribution, we tested the differences in “Twin” and “Control” dissimilarity by using the Wilcoxon test in R-software.

Results

The results of cluster analyses, as tested by PERMANOVA, confirmed our presumption and divided the dataset of all of the 14 relevés into two clusters, depending on the dominant tree – the cluster of black locust dominated forest plots and the cluster of native forest plots (Figure 2). The species composition of the understory vegetation differed significantly in this small area, whilst under the same environmental conditions, so the difference can be explained as an effect of the dominant tree species. All of the relevés from the native forest and all of the relevés from the black locust forest were merged at a much lower level of dissimilarity and both of the groups were fused at a distinctly higher level of dissimilarity. The results of the analyses also indicated that the native forest stands were more similar to each other than the stands of the black locust forest.

The Wilcoxon test proved that the dissimilarities of the twin plots were significantly different and higher than the “control” dissimilarities of the relevés within the same forest type. The same results were obtained between the “twin” and the black locust forest relevés and between the “twin” and the hardwood floodplain forest relevés (Figure 3). Therefore, we have concluded that the plots that were located in the same type of forest were significantly more similar than the closest plot that was located in the other type of forest.

Discussion

Over the last few decades, scientists have been interested in the impact of alien trees (black locust is common object of research) on the species composition of the understory vegetation.

There are various approaches used for an assessment of the impact of alien tree species on understory vegetation. One of these approaches is by using a big database (Dengler et al. 2011, Lososová et al. 2015, Berg et al. 2016)

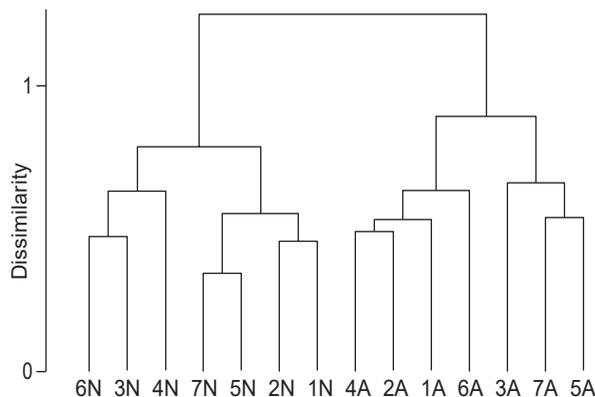


Figure 2: Result of hierarchical cluster analysis (SYN-TAX 2000, Hierclus). Two clusters represent native stands (N) on the left and alien black locust stands (A) on the right side. Used parameters: Bray-Curtis coefficient and β -flexible method ($\beta = -0.25$).

Slika 2: Rezultat hierarhične klastrske analize (SYN-TAX 2000, Hierclus). Dva klastra predstavljata naravne sestoje (N) na levi in sestoje tujerodne robinije (A) na desni. Uporabljeni parametri: koeficient Bray-Curtis in metoda β -flexible ($\beta = -0,25$).

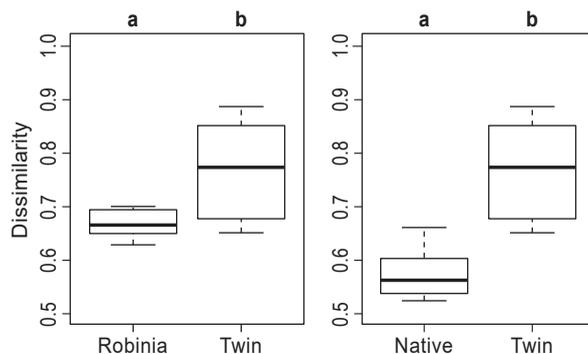


Figure 3: Comparison of the dissimilarity inside and between groups of relevés from same forest type and twin plots. For each relevé was calculated the “twin dissimilarity” – dissimilarity between current relevé and its related twin relevé from other forest type. Then the “control dissimilarity” was calculated as mean of dissimilarities between current relevé and all other relevés from the same forest type. The distribution of dissimilarities was tested using Wilcoxon test and the results are indicated by the markers of significance (a, b).

Slika 3: Primerjava različnosti znotraj in med skupinami popisov iz istega tipa gozda in dvojnih popisov. Za vsak popis smo izračunali “dvojno različnost” – različnost med popisom in povezanim dvojnimi popisom iz drugega gozdnega tipa. Nato smo izračunali “kontrolno različnost” kot povprečno različnost med popisom vsemi drugimi popisi iz enakega tipa gozda. Razporeditev različnosti smo testirali z Wilcoxonovim testom in rezultati so prikazani z oznakami statistične značilnosti (a, b).

or data from articles, where the cases of invasions have been published (Vilà et al. 2011). The advantage of this particular approach is a large dataset and easy data availability, but this data do not contain any ecological, environmental, or geological relations. By going this route, one can only obtain a rough image of the alien tree influence on the species composition of herbal synusia – and

the results are biased by different (or unknown) environmental conditions of compared groups. Furthermore, the dataset can also contain data which was sampled over a long period of time, even several decades. In another approach, the map of natural potential vegetation is taken into consideration (Chytrý et al. 2013, Bazalová et al. 2016). In this case, the relevés could have been selected on the basis of another ecologically specified area from different articles and databases (González-Muñoz et al. 2012, Woziwoda et al. 2014). However, in this approach, the abiotic factors are more or less not uniform on a local scale. The members of the relevés may not be close together. An unequal number of relevés in the compared groups is also a disadvantage of this method. Abiotic conditions (slope and the orientation of plots, geological base, and so on) are important factors, influencing the understory vegetation. These circumstances could all influence the evaluation of the results. The concept of twin plots has allowed for us to eliminate the disadvantages of the previous alternative methods.

The concept of twin plots was more widely used in the late 1990's by Walker & Smith (1997). They supposed that the twin relevés method increased the probability that the stands would have the same disturbance regime and land-use context. A neighbourly sampling system would guarantee similar geology, soil type, land-use, and climate. It is an acceptable approach, in order to study the effects of an alien tree species. The twin plots method has also been used in forest ecology, in order to determine nutrient limitations and the potential productivity of *Eucalyptus* L'Hér. plantations in Brazil (Stape et al. 2006).

There are several studies that have used the twin plots method. Benesperi et al. (2012) have claimed that under comparable environmental conditions in the Northern Apennines, the replacement of native forests (mixed oak, chestnut and European hop hornbeam forests) by pure black-locust stands, resulted in both a plant richness loss and a shift in species composition. The method of twin relevés was also used in an urban area of Berlin and there was a significantly lower alpha diversity in the black locust stands when compared with the native birch (Trentanovi et al. 2013). In the Czech Republic, Šenkýř (2015) proved that there was a negative impact of black locust, hybrid poplar, and black walnut, on the species richness and the composition of the understory, with a positive effect on the occurrence of alien species, when using the twin plots method. On the other side, in Northern Italy, result about the negative impact of young black locust trees (10-36 year old) on species diversity and composition was refuted (Sitzia et al. 2012) by using nearby paired sampling units. In addition, a study in

France chose a similar method of sampling relevés for an evaluation of the impact of alien tree species (Chabrierie et al. 2008). They supposed that a twin plots sampling approach reduced the impact of possible confounding environmental variables on the observed differences, in response to variables between the invaded and uninvaded stands. In a study about increasing the mineral nutrient in the soils under invasive plants in Belgium, this twin plots method was used, where patches of the targeted exotic plants were surrounded by uninvaded vegetation consisting of native species. Thus, site selection contributes to minimising the probability of pre-existing differences, prior to the invasion event (Vanderhoeven et al. 2005). The twin plots method was also used in a Canadian study, where it evaluated the effects of vegetation on soil resource heterogeneity on the prairie and in a forest (Kleb & Wilson 1997). The above-mentioned authors considered the twin plots method as being appropriate for assessing the impact of alien tree species on the species composition of the understory.

The basis of the twin plots method is that there is an assumption that the close proximity of the twin plots of natural and alien forests ensures a maximum similarity (uniformity) of their abiotic conditions. This fact has enabled this study to conclude that the differences in species composition of the understory are caused by the edifier (alien tree in the tree layer). We know from phytosociological practise that there are not even two relevés from forest vegetation which could be completely identical (not even relevés from the same forest community). Plots that have been recorded in the same forest are not identical and the differences in species composition of the understory are not dependent only on the tree edifier and abiotic factors, but natural variability as well. Natural variability is often the overlooked part in studies that have focused on the impact of alien tree species on the understory vegetation.

Those authors that have been using twin plots in order to evaluate the impact of alien species on diversity have not taken into consideration that the differences in species composition may not have been caused only by the impact of the alien species. Differences can partly be caused by the natural variability of vegetation and also by differences in the microclimatic conditions of the individual plots.

Our study has compared the dissimilarities within the relevés of one type of forest (native or alien) and between the twin relevés from different forest types. Our results of the cluster analyses, PERMANOVA, and the Wilcoxon test have shown that the differences in the species composition of relevés, from the same forest, are significantly lower than the differences between the twins.

Thus, the results of our analyses have suggested and proved that the twin plots method is a suitable tool for the assessment of the impact of dominant alien tree species on the species composition of the understory. The twin plots method eliminates the influences of the environmental factors (the same orientation, slope, altitude, geological base, and the soil type of twin plots) on the composition of understory vegetation and stresses the importance of tree edifiers.

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