Impacts of biological invasion and overwintering on spider fauna in plantation forest

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1. Introduction

The anthropogenic land use changes are resulted in habitat destruction and fragmentation worldwide, that is responsible highly for the loss of biological diversity. For instance, in Hungary, the area covered by forests is approximately 22.5% and is increasing by 0.5% per annum; however, 75% of the forests are primarily under intensive management focusing almost entirely on wood production. The expansion of tree plantations can have severe ecological impacts on native biota, such as alteration of plant and animal community compositions and ecosystem functioning. Although international pressure is increasing to tackle the negative environmental effects of such plantations, tree plantation covers more than 7% of total forest area worldwide. Deciduous tree plantations, such as poplar in central Europe, have dense understory vegetation similar to natural forests which can reduce extreme microclimatic conditions and favorable for forest specialist arthropods. However, in pine plantations, the floor is covered by pines needles, forming a homogeneous microhabitat structure.

The diversity of arthropods in plantations and native forests are already compared in numerous studies, but the winter activity of spiders in poplar and pines tree plantations has not been studied until now. Winter activity has several advantages including the ability to locate habitat, food resources. While prey availability is typically low in the winter, the competition is also reduced; furthermore feeding during winter can improve survival rates. The rate of successful overwintering is determined by body condition and is thus linked to prey availability in the overwintering habitat type. Large spiders may have higher chances of survival than small individuals of the same species, but this correlation is not always clear. Besides body size, numerous indices have been proposed for assessing the body condition of invertebrates. However, fat reserves in arthropods are considered good estimators of body condition, since age and nutrition intake determine the protein and lipid content of arthropods.

Transportation enhanced the occurrence and spread of non-native species. Biological invasion is also considered as serious threat for biodiversity and related ecosystem functioning. Most of the arthropods are closely associated with the native vegetation or its microhabitats. Alteration of habitat's physical characteristics has been responsible for negative consequences on the indigenous fauna. As native insects have different evolutionary history with exotic plants, it is quite difficult for them to get adapted for consumption of these plants as food resources. The effect of plant invasion on arthropod assemblage structure is still not well defined, and is crucial in understanding terrestrial ecosystem ecology.

The common milkweed (*Asclepias syriaca*) has become one of the most abundant invasive plant species in Hungarian lowland forest plantations, and represents a major problem in conservation areas. *A. syriaca* attracts many insects, particularly pollinators, because of the open structure of its flowers. As such, it serves as a continuous resource for pollinators. The high density of pollinators, in turn, may attract predatory arthropods.

Traditionally, species richness or taxonomic diversity has been considered as one of the most commonly used indices of biodiversity. The functional diversity approach offers a useful tool to assess ecosystem functions and services. Numerous studies showed that increasing species richness and diversity can enhance ecosystem functioning; however, large part of the variation may remain unexplained. The functional diversity of arthropods in semi natural habitats is still not studied deeply. With this, increase in overall functional diversity in the plantation forests may enhance resistance and resilience of ecosystems.

2. Aims and objectives

The main aim of my work was to reveal how plantation forest tree species, plant invasions in plantation forests and overwintering affects spiders.

The knowledge gap

The effect of habitat structure of forests on spiders has been documented; however, the majority of this work focuses on species diversity patterns, with few studies focusing on functional diversity of spiders. Furthermore, there is limited information on how arthropod assemblages and functional diversity is affected by plant invasion in different forest types and how forest type affects winter active spiders. Furthermore, the effects of overwintering on spider body condition and behaviour in plantation forests have not yet been studied.

The present dissertation covers three studies to fill the above knowledge gaps

(1.) In first study we assessed the effect of *A. syriaca* invasion on species richness, and species composition of spiders in the poplar and pine plantation forests. We also applied the functional diversity concept to link diversity patterns with ecosystem processes and functioning.

(2.) The aim of second part of my thesis was to reveal the differences in the species richness and community composition of spiders in the poplar and pine plantation during winter.

(3.) Finally, we aimed to test the effects of winter on the behaviour and body condition of spiders collected from poplar and pine plantations.

3. Materials and methods

3.1 Study area

All studies were carried out in the Kiskunság region, in the southern part of the Great Hungarian Plain (46° 42 ′ N, 19° 36 ′ E). The dominant land use types are plantation forests and agricultural land. The landscape of the Southern Kiskunság region is dominated by different aged plantation forests under intensive management. These secondary forests include coniferous and deciduous tree plantations

3.2 Effect of *A. syriaca* and different tree species

3.2.1 Study design and sampling

Sites (N=40) were selected according to tree species (native poplar forests vs. exotic pine plantations) and common milkweed density (invaded vs. non-invaded sites) in a full factorial design resulting in 10 replicates per treatment combination. We collected spiders with pitfall traps.

3.2.2. Data analysis

From the habitat structure data, mean values were calculated for each variable at each site. To detect possible differences in herbaceous cover, average height of the vegetation and the cover of leaf litter, the above parameters were assessed in 1×1 meter quadrates, from the habitat structure data, mean values were calculated for each variable at each site.

We classified spider species according to their shading tolerance, moisture preference, feeding, and size. We calculated community-weighted mean (CWM) values for each trait at each sampling site; functional dispersion (FDis) and Rao’s quadratic entropy (RaoQ) to characterize the functional diversity of spider assemblages. We applied generalized linear mixed models (GLMMs) where forest type (i.e., poplar vs. pine), presence of *A. syriaca* (i.e. invaded vs. non-invaded sites) were fixed factors. Sampling site nested in plantation forest was used as random effect.

Functional diversity measures, species richness and activity density data were our dependent variables.

We explored the multivariate response of spider assemblages to tree species and the presence of *A. syriaca* with non-metric multidimensional scaling (NMDS) and non-metric multivariate ANOVA (NPMANOVA). Furthermore, we used indicator value analysis to detect characteristic spider species.

3.3. Effect of tree species on winter fauna

3.3.1 Study design and sampling

We used the sampling sites described in section 3.2 with the same collecting method and same arrangement of traps in two consecutive winters for three one-month sampling periods of 2017-2019. We characterized the habitat structure at the sampling sites by the percentage cover of leaf litter, mosses, herbaceous vegetation and shrubs. We assessed the microclimatic differences using data loggers (Optin ADL TH3-32) by recording hourly temperature data.

3.3.2 Data analysis

We determined the effect of forest type on habitat structural characteristics (percentage cover of leaf litter, mosses, herbaceous vegetation and shrubs) by using linear mixed effect models. We used plantation type (pine or native) as fixed factor, whereas spatial distribution of sampling sites (forest ID) was considered as a random factor. We determined the effect of forest type and habitat structure on species richness, adult activity density and total number of collected spiders, including juveniles, using generalized linear mixed effect models. Plantation type and habitat structural characteristics with no significant correlation with plantation type were used as fixed factors and forest ID was considered as a random factor.

We explored the multivariate response of winter active spider assemblages to tree species with the help of non-metric multidimensional scaling (NMDS) and non-metric multivariate ANOVA (NPMANOVA). We used indicator value analysis to identify the characteristic species of spiders in pine and poplar forests.

3.4. Effect of forest type on spider behaviour and condition

3.4.1 Study design and sampling

We studied the wolf spider *Pardosa alacris* C.L. Koch, 1833 (Lyosidae, Araneae), a very common species in central European forests. The specimens used in the present study were collected in six forest plantations (three poplar and three pine) using the ground hand collecting method. We collected 15 sub-adult *P. alacris* before overwintering of 2016, and after winter of 2017, in each of the sampling sites.

3.4.2 Locomotory behaviour trials

We studied the cursorial locomotion of *P. alacris* by tracking and recording their movements in a white circular plastic arena. The locomotory activity was determined by digitalising their path using the SMART video tracking software to measure (1) total distance travelled (cm); (2) total moving time during the 180 s of observation (s); (3) average speed of the individuals when moving (cm/s) was calculated with the help of (1) and (2).

3.4.3 Body size and fat percentage

Spiders were fixed by freezing at -15°C. The gender of the spiders was identified, and the total body length and prosoma length of spiders were measured. Spiders were subsequently dried at 60°C for four days and then we recorded dry weight. The spiders were then moved to glass vials, and the fat was extracted with 4 ml petroleum-ether for six days. Spiders were subsequently dried again at 60°C for four days and their weight was measured. The fat proportions of spiders were calculated by the ratio of final mass and dry mass.

3.4.4 Data analysis

To test if season (autumn/spring), forest type (poplar/pine), gender and their second order interaction had significant effects on the size, fat content and moving behaviour of *P. alacris* spiders, we used mixed effect linear models and model averaging. We also added percentage fat content as a fixed effect in the models where total distance covered and speed were the dependent variables, and the random effect term was “forest ID”. However, for total distance covered and speed models, sex was used as a random effect instead of fixed effect, in order to control for potential multicollinearity between gender specific locomotory activity and sex. All possible linear combinations of the above fixed effects were considered and ranked according to Akaike’s information criteria corrected for small sample sizes (AICc) The models with <10 ΔAICc of the best model (i.e. the model with the lowest AICc) were used for model averaging.

4. Results

4.1. Effect of *A. syriaca* and tree species

We collected 1621 adult spider specimens from 53 species.

We did not find a significant effect of tree species or *A. syriaca* invasion on the species richness and abundance of spider assemblages. There was a significant effect of *A. syriaca* on FDis and RaoQ of spiders, with the invaded sites having lower functional diversity than non-invaded sites. The significant interaction effect of forest types and invasion of *A. syriaca* on FDis and RaoQ of spiders indicated that invasion had a more pronounced effect in pine than in poplar forests. Spider species were larger and web building spiders were more abundant in poplar forests than in pine plantations.

Spider assemblages of the two forest types clearly separated according to the NMDS and Non-metric multivariate ANOVA, with numerous species associated with poplar or pine plantation.

4.2. Effect of tree species on winter fauna

We collected 1,337 spiders, out of which 735 were adults from 26 species.

Pine plantations maintained a more temperate microclimate than poplar plantations. Percentage cover of leaf litter and mosses were positively related with pine plantations. We found higher species richness and activity density in pine plantations than in the poplar forests. Percentage coverage of shrubs had a significant negative effect on adult spider activity density; however, we found no significant effect of herbaceous vegetation cover.

The species composition of spiders in pine and poplar plantations differed significantly according to the PERMANOVA. However, NMDS plot showed that species composition of pine plantations were less variable than that of the poplar plantations. We identified 6 and 1 significant indicator species for pine and poplar forests, respectively. All of the indicator species in pine plantations preferred humid habitats; however, poplar forest indicator species preferred dry and partly shaded habitats.

4.3. Effect of forest type on spider *P. alacris* behaviour and condition

Subadult female spiders were larger than subadult males and subadult spiders collected from poplar forests were smaller than from pine forests. We found a significant interaction effect of forest type and season on body length, suggesting a more pronounced effect in poplar forests than in pine forests. Furthermore, the significant interaction effect of season and gender indicated that the difference between body length in autumn and spring in subadult males was greater than that of the subadult females in autumn and spring, respectively.

The percentage of fat content was significantly higher in spring spiders than in autumn spiders. Total distance covered and speed was significantly lower in spring spiders than in autumn spiders. Spiders with higher fat content moved slower.

5. Discussion

5.1. Effect of *A. syriaca* and tree species

We found different species compositions for poplar and pine forests. Furthermore, we found a higher proportion of web-building spiders in poplar forests than in pine forests. The architecture of forest canopy is formed by presence of tree elements and gaps can strongly influences the solar radiation. Forest canopy opening is major driver of community structure of ground-dwelling invertebrates. Canopy closure can affect the soil microclimate and understory vegetation development. Complex vegetation structure might offer plentiful prospective of the web attachments for web-building spider species.

We found a higher proportion of larger species in poplar forests than in pine forests. The abundance of litter-dwelling invertebrates is expected to be related to the amount of leaf litter, as well. The leaf litter in pine plantations consists of pines needles which reduces soil pH and may change the physical properties of the soil. Spider diversity and density is mainly organized bottom-up forces in food webs. The major part of diet of ground-dwelling spiders in forests is Collembola, which are abundant and diverse in forest soils. In the present study, we also found larger CWM size values in poplar forests than in pine forests.

The thick moss-litter layer, shrub-rich understory vegetation, and coarse woody debris together with variation in light regimes due to the gaps in dense canopy layer may determine spider assemblages directly and via prey abundance. Dense vegetation offers several micro-habitats, which have an important role in regulating the species composition of spider assemblages. The relatively uniform microhabitat conditions may result in a uniform spider species composition. In this study, we found that species composition differed between forest types, as indicated by the significant results of multivariate ANOVA and the clear separation by NMDS ordination. The high number of significant indicator species also underpinned the marked differences in spider assemblages of pine and poplar forests, even though we detected no differences in herbaceous vegetation cover between the plantations types. However, the species composition differs and diversity of vegetation is lower in pine than in poplar plantations.

However, the functional diversity was higher in non-invaded sites than in invaded sites; we found no effect of *A. syriaca* invasion on the abundance and species richness of spiders. *A. syriaca* had a negative effect on functional diversity in pine forests, while its effect was less pronounced in poplar forests. Invasive plants may offer novel food resources to various native herbivore arthropods, which can affect the potential prey abundance for spiders. The non-invaded sites in pine plantations had similar species richness and higher functional diversity (FDis and RaoQ indices) which suggested that traits values are less similar than in invaded sites. In contrast, the invaded sites had lower functional diversity, and thus a uniform trait state composition. Invaded pine forests only preferred a certain trait state combination, which implied that environmental filtering played an important role in species sorting.

5.2. Effect of tree species on winter fauna

We found higher species richness in pine plantations than in the poplar plantations. However, the spider species composition of winter-active communities is different in poplar and pine plantations.

Microclimatic factors, such as temperature and moisture have an effect on species richness and activity density of spiders. Species composition and richness of trees of forests can also affect the spider richness, abundance and community composition. As poplar is deciduous tree, it drops the leaves before the winter and it results in higher solar transmission in the poplar plantation than in the pine plantations during the winter. This condition may not maintain the uniform temperature. Thick canopy in the pine plantations can provide thermal insulation effect throughout day and night and maintained the more temperate microclimate. This may provide possibility of survival to many spider species and results in higher species richness in pine plantation than in poplar plantation in the winter Poplar plantations may provide higher quality habitats for the spiders than pine plantations. Such complex habitat structures are expected provide variety of prey organisms in a large abundance. For web builders, it can provide more attaching points for webs and shelters. Percentage coverage of shrubs had a significant negative effect on activity density of spiders; however, we found no significant effect of herbaceous vegetation cover on winter active spiders.

Presumably, the effects of microclimatic parameters overrode the effect of microhabitat heterogeneity.

We also identified significant indicator spider species for both plantations. Soil and litter moisture affect the composition of spider communities during autumn. Wind flow together with canopy closure in the forests can determine the moisture of the forest floor. The layer of litter may also regulate the moisture level of soil indirectly by reducing capillary rise and evaporation. The moisture condition in pine plantations and in poplar plantations are different which may also contribute to the differences in spider species composition. This can result in a higher species richness of spiders in pine forest than in poplar forest. Several collected species of the family Linyphiidae with a preference for moisture and shading (e.g., *Cenromerus sylvaticus*, *Ceratinalla brevis* and *Walckenaeria alticeps*) were found as significant indicator species of pine forests.

5.3. Effect of forest type on spider behaviour and condition

We analyzed the effect of forest type and overwintering on body condition and locomotory activity of the diurnal wolf spider *P. alacris*. We found that overwintering had significant positive effect on the fat reserves of spiders; however, it had no effect on the body length of *P. alacris*.

We collected smaller spiders in the poplar forests than in the pine plantations, and the significant interaction effect indicated that the difference in body length of autumn and spring spiders was more pronounced in poplar forests than in pine plantations. Warmer condition in deciduous forests may advantageous for foraging activity of spiders.

We also found a significant positive interaction effect of season and forest type, and season and gender on the length of spiders, indicating that the effect of overwintering is mediated by habitat type and gender. We collected larger subadult female than subadult male spiders, and the difference between body length of subadult males in autumn and spring was greater than that of subadult females. The larger body size in female spiders may result in higher reproductive success through a fecundity advantage as female fecundity changes with mass. Spiders collected in spring had greater fat content than spiders collected in autumn. Larger size of body might be gained through fast growth rate or by prolonging the growth period. At high quality habitats spiders forage more and reach adult body size faster. In the temperate zone many spider species forage in winter days prolonging their growth period.

Spiders collected in spring moved less and moved slower than spiders collected in autumn and spiders with high fat content moved slower. We found that the spiders from poplar plantations were smaller than in pine forests. Furthermore, we found that in poplar forests overwintering had a more positive effect on body size than in pine plantations. The spiders with good body condition might have a higher rate of survival and successful overwintering. Intraguild predation is very common mortality factor for wolf spiders that may result in reduction of movement speed of *Pardosa* species in the presence of larger spiders. We also found that spring spiders were slower than autumn spiders and they covered less distances. However, increased immobility duration during winter may results in reduction of feeding efficiency and further may be responsible for weight loss. Avian predation risk in early spring nesting bird species, may have in indirect negatively affect spider speed and mobility during spring.

6. Conclusion

In conclusion, plantation type and invasion of *A. syriaca* affected different elements of functional diversity of spiders. The information on the effect of pine plantations and *A. syriaca* invasion on biodiversity is critical for forestry and conservation management.

We also conclude that the effect of microclimatic differences and prey availability presumably overrides the effect of habitat structure on winter-active spiders. In the conclusion of third part of our study, before winter, spiders in poplar forests were smaller than spiders of pine forests, but during winter, spiders in poplar forests grew more than spiders in pine forests.

List of articles on thesis (MTMT No: 10070640)

1. Ingle, K., Gallé-Szpisjak, N., Kaur, H., & Gallé, R. (2019). Forest type interacts with milkweed invasion to affect spider communities. *Insect Conservation and Diversity*, *12*, 321–328. **(IF- 2.92)**
2. Ingle, K., Horváth, Á., Gallé-Szpisjak, N., Gellért, L., Csata, E., & Gallé, R. (2018). The effects of overwintering and habitat type on body condition and locomotion of the wolf spider Pardosa alacris. *Acta Oecologica*, *89*, 38–42. **(IF 1.65)**
3. Ingle, K., Kaur, H., Gallé-Szpisjak, N., Bürgés, J., Szabó, Á., & Gallé, R. (2020). Winter-active spider fauna is affected by plantation forest type. *Environmental Entomology*, *49*, 601–606. **(IF- 1.58)**

Other Articles

4. Gallé, R., Császár, P., Makra, T., Gallé-Szpisjak, N., Ladányi, Z., Torma, A., Ingle, K., Szilassi, P. (2018). Small-scale agricultural landscapes promote spider and ground beetle densities by offering suitable overwintering sites. *Landscape Ecology*, *33*, 1435–1446. **(IF- 4.51)**
5. Ingle, K., Vitkin, E., Robin, A., Yakhini, Z., Mishori, D., & Golberg, A. (2018). Macroalgae biorefinery from Kappaphycus alvarezii: conversion modeling and performance prediction for India and Philippines as examples. *BioEnergy Research*, *11*, 22–32. **(IF- 2.58)**
6. Ingle, K., Polikovsky, M., Chemodanov, A., & Golberg, A. (2018). Marine integrated pest management (MIPM) approach for sustainable seagriculture. *Algal Research*, *29*, 223–232. **(IF- 3.99)**
7. Ingle, K., Traugott, H., & Golberg, A. (2020). Challenges for marine macroalgal biomass production in indian coastal waters. *Botanica Marina*, *1*, 1–15. **(IF- 1.38)**