# The Community Structure of Ants in *Hordeum Vulgare* and Grass Mixture Conditions in the Southwestern Part of Slovakia

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## ABSTRACT

Ants are an important bioindicative group that plays a significant role in agroecosystems. As a result of interspecific competition for food, they can displace native species. The aim of the research was to assess the influence of environmental variables (soil pH, soil moisture, potassium, phosphorus and nitrogen) and the influence of seasons on the dispersion of ants. Between 2018 and 2020, while investigating different types of crops, we recorded 864 individuals belonging to 9 species and 2 unspecified species (sp.). The dispersion of ants was affected by moisture, soil pH, phosphorus, potassium and nitrogen. In addition, an increase in value of the average number of individuals during spring and summer months was confirmed. We confirmed an increasing number of ant individuals with increasing values of potassium, phosphorus, nitrogen and soil moisture. A neutral pH of soil is optimal for ants. Our results yielded new information indicating that agricultural intensification negatively affects ants which are important for the production of biomass and reduces the number of pests which also affect crop yields.

Keywords: ants, abundance, agrosystems, diversity, field margins.

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## INTRODUCTION

In a world with a rapidly expanding population of people, there is a growing demand for food and a simultaneous need for higher environmental sustainability. The area available for agricultural production is limited, and methods which do not compromise vields are needed. Insects are one of the largest groups of animals which play a vital part in the conservation of ecosystems, improve the health of an ecosystem, and are the critical component in the food web in both terrestrial and aquatic ecosystems (Courtney, 1994; Brygadyrenko, 2015; Faly, Kolombar, Prokopenko, Pakhomov, & Brygadyrenko, 2017; Avtaeva, Sukhodolskaya, & Brygadyrenko, 2021). Ants are one of the most ecologically dominant groups of insects in terrestrial habitats. Their ecological success can be attributed to the variety and efficiency of their foraging habits, eusocial mode of life, local abundance and the ability to adjust their activity to environmental changes (Rongue, Fourcassié, & Oliveira, 2018). They are a social insect group that carry out various roles such as predator, prey, detritivore and herbivore (Diamé, Rey, Vayssières, Grechi, Chailleux, & Diarra 2018). They vary significantly, there are almost 14,000 species found widely distributed across the earth (Bolton, 2021). They are cosmopolitan and exist across several different ecosystems, including forests, damp places, water sources and drylands. Given this presence, it is no wonder that ant diversity is used as a bioindicator to determine ecosystem and environmental changes (Gibb, et al, 2020; Oberprieler & Andersen, 2020).

Ants contribute to various ecosystem services including soil dynamics and nutrient cycling, they directly affect species composition in animal and plant communities (Toro, Ribbons, & Pelini, 2012), they represent an important component of agricultural ecosystems (Offenberg, 2015) and especially semi-natural habitats within agricultural landscapes (Marshall & Moonen, 2002). They are skilful tillers of soil, dispensers of seeds and microbial propagules, transmitters of N2-fixing bacteria, ecosystem engineers, fungi growers, waste managers, biotechnologists, pest controllers, soldiers and reproducers (Benckiser, 2007). Their densities and compositions in agricultural sites depend on human activities and are predictable in a typical agricultural land-use mosaic such as arable, fallow, grassland fields and forest sites (Dauber, 2001; Braschler, 2005; Purkart, Kollár, & Goffová, 2019). Therefore, many ant species hold desirable characteristics unshared by most other beneficials. They comprise at least one-third of all insect biomass (Hölldobler & Wilson, 2009). With such abundance, any interaction derived from this taxon holds a high potential. Most ant species are polyphagous, cooperative often with polymorphic worker forces, enabling them to deploy a wide range of prey types. They may exert pressure on several pest species and their life stages. Their territorial behaviour makes them attack and deter pests that are far beyond the size of potential prey (Manak, Nordenhem, Bjorklund, Lenoir, & Nordlander, 2013). Weaver ants (Oecophylla smaragdina and O. longinoda) control more than 50 different pests in 12 different crops. They are able to increase farmers' net income by more than 70% when substituting conventional pesticide regimes (Peng, Christian, & Gibb, 2004; Peng & Christian, 2005). Ants' predation makes them prospects for future integrated pest management strategies in agriculture (Offenberg, 2014).

The objective of this study was to analyse the community structure of ants in the conditions of *Hordeum vulg*are and *Grass mixture*. In the work, we also analysed the effects of environmental variables (soil humidity, soil pH, potassium, phosphorus and nitrogen), which might influence their abundance and population structure. The results of our work might influence for the correct setting of crop management, so that there is no disturbance to the population of ants due to their importance in agroecosystems, where they are part of the biomass and also participate in the reduction of crop pests.

## MATERIALS AND METHODS

The research took place in the year 2018 to 2020 and we collected ants in two types of agricultural crops. In the winter crop of *Hordeum vulgare*, ants were collected from November to July. In the *Grass mixture*, ants were collected year-round. These types of agricultural crops were examined throughout each year, the position of crops in the fields changed every year (Klimánek, 2008). Crops were grown in a conventional way. The soil was ploughed three times and turned. Pre-sowing preparation and sowing were combined. Machines with driven working tools were used in conjunction with a seed drill. When sowing, it was possible to use seed coulters with an obtuse angle of penetration into the soil.

#### Study area

The study area of agricultural crops is located in the geomorphological unit of the Podunajská pahorkatina - the Danubian upland (the south-western part of Slovakia) in the cadastral territory of Nitra Fig. 1. The altitude of the monitored area was approximately 130m above sea level with a brown type of soil. The study area belongs to a warm arid climate area with mild winters (Table 1).



Figure 1. Map of the study area.

Month	Average temperature (°C)	Average rainfall (mm)
January	-5-5	30
February	-3-6	26
March	0–12	35
April	10–20	12
May	15–22	65
June	18–27	77
July	22–29	41
August	20–29	57
September	15–23	64
October	8–15	54
November	-3-7	40
December	-5-5	55

Table 1. Average values of temperature and rainfall.

#### Collection of samples and application of sprays

We used five pitfall traps for each site, which were placed in a line at a distance of 10 metres. A 4% formaldehyde solution to fix the material was used. Pitfall traps were always in the fields and were collected at two-week intervals. The nomenclature and determination of ants was established according to the work of Seifert (2018).

The insecticide FORCE (Syngenta, Basel, Switzerland), a granular insecticide in-tended for soil application to control soil pests, was applied to the crops. Insects were killed through respiratory and tactile poison ingestion. The preparation had a fast effect and a strong residual (repellent) action against a wide range of soil pests from the orders of Coleoptera, Aranea and Hymenoptera. The applied dose was administered uniformly at a concentration of 12–15 kg per ha each year for the duration of the research. Solinure FX fertiliser (Medilco Hellas S.A., Athens, Greece) containing chlorides and urea, was applied to the crops and was intended for field fertility. Due to its acidifying effect, it contributed to lowering the soil pH.

#### Measurement of environmental variables

At each pitfall trap location we removed stones and fallen leaves from crops and sampled the soil to a depth of 15 cm for analysis. Five samples (one from each of five sites) were taken from each field every two weeks over the three years of the study period. Subsequently, environmental variables (N, P, K, pH, moisture) were analysed using a soil moisture meter (Rapitest 3 1835, Luster Leaf, Illinois, IL, USA) and a pH meter (Dexxer PH-03, Luboň, Poland). We thoroughly wetted the broken up soil with water (ideally distilled or deionised water) to a muddy consistency. We wiped the meter probe clean with a tissue or paper towel and inserted it into the soil up to the probe base (7-10 cm). We waited one minute and wrote down the value. We converted the measured values into units of mg.

#### **Database quality**

The data obtained by research has been saved in the Microsoft SQL Server 2017 database program (Express Edition), consisting of frequency tables for collections and measured environmental variables, (pH, soil moisture, potassium, phosphorus

and nitrogen). The database also consisted of code tables for study sites and their variables (crops, habitat, locality name, cadastral area, altitude and coordinates of localities). Matrices for statistical calculations using the Microsoft SQL Server 2017 were programmed.

#### Statistical analyses

The multivariate analysis (redundancy analysis - RDA) to determine the dependencies between objects (ants, agricultural crops and soil characteristics) was used. We tested the statistical significance of pH, soil moisture, potassium, phosphorus and nitrogen with the Monte Carlo permutation test in the CANOCO5 program (Ter Braak & Šmilauer, 2012).

Analysis in the statistical program Statistica Cz. (StatSoft Inc., 2004) focused on polynomial regression, expressing the relationship between the number of ants and the values of potassium, phosphorus, nitrogen, pH and soil moisture. The Shapiro-Wilk W-test determined the normality of data distribution. Based on the violation of the normality data distribution (p-value = 0.00), we used the nonparametric Friedman test (ANOVA). It was used to test the differences in the number of individuals between the months.

## RESULTS

Over a period of three years of research, we found a total of 864 individuals belonging to 9 species and 2 unspecified species (sp.) in the studied area. Species of *Lasius niger* (83.80%) and *Tetramorium caespitum* (11.11%) had a eudominant representation of individuals, other species had subdominant to subrecendent representation (Table 2).

Species	Grass mixture	Hordeum vulgare	∑ ind.
Formica cunicularia Latreille, 1798	4	11	15
Formica rufibarbis Fabricius, 1793	0	2	2
Formica sp.	0	1	1
Lasius alienus Förster, 1850	0	1	1
Lasius niger (Linné, 1758)	305	419	724
Lasius sp.	3	3	6
Lasius umbratus (Nylander, 1846)	2	11	13
Myrmica sabuleti Meinert, 1861	0	4	4
Polyergus rufescens (Latreille, 1798)	1	0	1
Solenopsis fugax Latreille, 1798	1	0	1
Tetramorium caespitum Santschi, 1927	1	95	96
∑ individuals	317	547	864

Table 2. Distribution of the ants in the agricultural crops during the years 2018 - 2020.

Multivariate analysis of the ants between the years 2018 and 2020 was determined using the redundancy analysis (RDA, SD = 1.40 on the first ordination axis). We observed the relationship between ants and environmental variables (pH of the soil, soil moisture, potassium, phosphorus and nitrogen). The values of the explained variability of taxonomic data were 50.9% on the first ordination axis and 54.8% on the second

ordination axis. The cumulative variability of the species set explained by environment variables was represented in the first ordination axis 88.9% and in the 2nd axis 95.7%. Using the Monte Carlo permutation test, we identified a statistically significant effect of soil moisture (p = 0.0088, F(1.0276) = 2.0021, df = 5), soil pH (p = 0.0508, F(1.1183) = 1.9297, df = 5), phosphorus (p = 0.0466, F(1.1952) = 2.0805, df = 5), potassium (p = 0.0328, F(1.7145) = 1.9620, df = 5) and nitrogen (p = 0.0490, F(1.7006) = 2.1005, df = 5) on the structure of arthropods. The selected variables were not mutually correlated with the maximum value of the inflation factor = 4.3243. The ordination graph (triplot) contained ants ordered into one cluster (Fig. 2). The first cluster (I) consisted of ants correlated with phosphorus (mg) and moisture. The *Formica rufibarbis* species has links to potassium (mg) and soil pH. *Polyergus rufescens* correlated with nitrogen (mg). *Solenopsis fugax* was not affected by environmental variables.

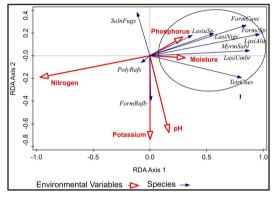


Figure 2. RDA analysis of ants with environmental variables.

The normality data distribution (number of individuals) was violated (p-value = 0.00). Based on that, a nonparametric Friedman test (ANOVA) was used to confirm the statistically significant difference (p-value = 0.04670, F (2.27) = 2.83040, df = 3) (Fig. 3) of individuals between months and crops of the *Hordeum vulgare* and *Grass mixture*. The results showed an increase in the average value of individuals in June - August in the crops *Hordeum vulgare*. Under *Grass mixture* conditions, the number of individuals increased from March to June and decreased in the following months.

The number of individual ants was processed using polynomial regression. Using the regression model, we expressed the relationship (correlation) between the number of individuals of ants and potassium (mg), phosphorus (mg), nitrogen (mg), pH and humidity (%). The correlation coefficient value was high for the number of individuals and pH (r = 0.8140) (Fig. 4, A), potassium (r = 0.9012) (Fig. 4, B), phosphorus (r = 0.8905) (Fig. 4, C), nitrogen (r = 0.7981) (Fig. 4, D) and moisture (r = 0.881) (Fig. 4, E), which indicated a strong relationship. The reliability coefficient for the pH r2 = 0.6899 indicated the capture of 68% variability, potassium r2 = 0.6908 (69% variability), phosphorus r2 = 0.7504 (75% variability), nitrogen r2 = 0.7145 (71% variability) and moisture r2 = 0.7384 (73% variability). The overall suitability of the regression model is statistically significant in all cases: pH (p-value = 0.0015),

potassium (p-value = 0.0428), phosphorus (p-value = 0.0298), nitrogen (p-value = 0.0248) and moisture (p-value = 0.0118). The results showed that increasing values of potassium, phosphorus, nitrogen and soil humidity also increased the number of ant individuals. The ideal value for ants was 16 - 22 mg/kg potassium, 1.3 - 1.8 mg/ kg phosphorus, 16 - 22 mg/kg nitrogen, 7 pH and 14 - 22 % for moisture.

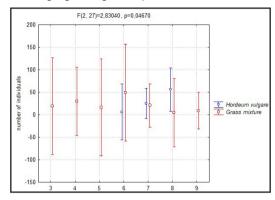


Figure 3. Friedman test (ANOVA) difference in the number of individuals between month and crops.

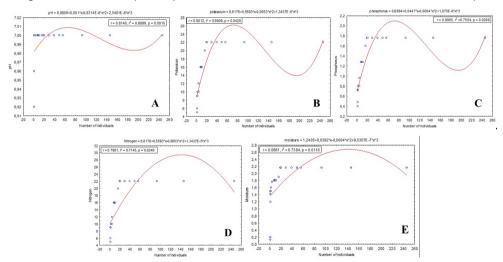


Figure 4. Polynomial regression model potassium, phosphorus, nitrogen, pH and moisture on the number of individuals of the ants.

## DISCUSSION

Ants living in agricultural landscapes have a wider tolerance than ants from natural habitats. They can achieve high local density due to the influence of agriculture and field margins support the most diverse community of ants (Bote & Romero, 2012; Oliveira et al, 2012; Magura, Ferrante, & Lövei 2020). We recorded that the ant community was

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dominated by species *Lasius niger* and *Tetramorium caespitum*. The great abundance of ants affects the maintenance of the natural balance and substance cycle of the biogenic elements in ecosystems such as carbon, nitrogen, sulphur and phosphorus. The dominance of Hymenoptera (Formicidae) and Coleoptera has been indicated as a general trait of ground-dwelling assemblages (Miranda, Piñero, & Megías, 2007; Lenoir & Lennartsson, 2010; Pardee & Philpott, 2011). Their activities accelerated the decomposition of plant residues, aerated the soil and improved soil structure and quality (Holecová, Lukáš, & Harakaľová, 2003; Dieng, Ndiaye, & Taylor, 2016). The dominant representation of the ants (Formicidae) and Coleoptera taxon among epigeic arthropods in the conditions of integrated farming and ecological farming was also recorded by Porhajašová Noskovič, Rakovská, Babošová, & Čeryová (2015); Porhajašová, Babošová, Noskovič, & Ondrišík (2018).

Exploiting biodiversity on ecosystem service provision is a goal of contemporary agriculture, although relationships between diversity and ecosystem services remain largely unexplored for innovative practices (Kalivoda, Petrovič, & Kürthy, 2010; Finney & Kave, 2016; Griffiths et al. 2000; Špulerová, Petrovič, Mederly, Moises, & Izakovičová 2018; Dobrovodska, Kanka, & David, 2019). Ants play an irreplaceable role in the decomposition of organic matter, in the cycle of biogenic elements of carbon, nitrogen, sulphur, phosphorus, in transformation and degradation of waste and toxic substances, and their presence is irreplaceable (Fazekašová & Bobulovská, 2012). Using the multivariate model, we demonstrated the influence of environmental variables (pH of the soil, soil moisture, potassium, phosphorus, nitrogen) on the abundance of ants. Thus, our results agreed with the results of (Attwood, Maron, House, & Zammit (2008)), who observed a change of abundance of ants with increasing land use. Biodiversity loss as a consequence of agricultural intensification can lead to reductions in agroecosystem functions and services. Increasing crop diversity through rotation may alleviate these negative consequences by restoring positive interactions. The impact of ants is an important component of the strategy leading to the sustainability of the soil ecosystem. The diversity of ants, including its abundance in soil, depends on the abiotic and biotic factors that are typical of the biotope (Zak, Holmes, White, Peacock, & Tilman, 2003; Tiemann, Grandy, Atkinson, Spiotta, & McDaniel, 2015).

Arthropod abundance from month to month is usually interpreted as being related to fluctuations in climatic factors (such as temperature, precipitation and day length) (Lionello, Rizzoli, & Boscolo, 2006). The number of ants in March and June was higher from the number of ants captured in July and September in *Grass mixture* conditions. In the *Hordeum vulgare* crop we have seen a steady increase. Simão, Carretero, Amaral, Soares, & Mateos (2015) also confirmed differences in the number of ants affected by different weather during the seasons. Andrew, Roberts, Hill (2012) have suggested that precipitation is more influential on ant diversity at high temperatures than at low temperatures. Greenberg & McGrane (1996); Majeed, Rana, Azevedo, Elmo, & Nargis (2020) confirmed a seasonal trend for the abundance of arthropod groups. It is established that environmental variables and the influence of biogeographic factors account for fluctuations in species abundance. Climatic

conditions during the months impact the biodiversity of ant species (Garcia, Cabeza, Rahbek, & Araújo, 2014; Williams & Newbold, 2020). In our study, we confirmed with the help of regression models a strong relationship between the environmental variables potassium (mg), phosphorus (mg), nitrogen (mg), pH, humidity (%) and the abundance of ants. For agricultural management, understanding how species' behaviour varies with environmental variables is imperative in ensuring food security in the future. In addition, ants' predation makes them prospects for future integrated pest management strategies in agriculture (Offenberg, 2014). Ants mineralize nutrients, form soil aggregates, and disperse seeds, are significant and necessary for decorous ecosystem functioning and sustainability (Del Toro, Ribbons, & Pelini, 2012; Pfeiffer, Mezger, & Dyckmans 2013).

## CONCLUSION

Our results have provided new knowledge about the preference of ants in the conditions of *Hordeum vulgare* and *Grass mixture* in central Europe. The dispersion of ants was influenced by soil moisture, soil pH, phosphorus, potassium and nitrogen. We confirmed an increase in the average number of individuals during spring and summer months. The ants had a strong correlation with soil moisture (%), soil pH, phosphorus (mg/kg), potassium (mg/kg) and nitrogen (mg/kg). With increasing values of potassium, phosphorus, nitrogen and moisture, the number of individuals also increased. We confirmed that the optimal soil pH value was neutral. A practical, workable approach should be used to preserve the current ant population, achieve sustainable levels of biodiversity, key species to develop conservation and agricultural management strategies. This is of particular importance to those who may face pressure from pest species threatening crop yields. This study can be helpful in the planning of conservation programs as well as provide information to farmers to initiate integrated pest management strategies.

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