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Ekológia (Bratislava) Vol. 38, No. 2, p. 101–116, 2019
DOI:10.2478/eko-2019-0008

THE CONTINGENCY OF SOIL MICROORGANISMS AND THE SELECTED SOIL BIOTIC AND ABIOTIC PARAMETERS UNDER DIFFERENT LAND-USES

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Abstract

Júdová J., Kanianska R., Jaďuďová J., Kizeková M., Makovníková J.: The contingency of soil microorganisms and the selected soil biotic and abiotic parameters under different land-uses. Ekológia (Bratislava), Vol. 38, No. 2, p. 101–116, 2019.

Land use changes are local phenomena with global impact. They have an impact in a cumulative sense on biodiversity or soil degradation. This study aimed to examine the effects of different land-uses (arable land, permanent grasslands, abandoned grasslands, forest land) on the selected biotic and abiotic soil parameters in the Slovak mountain study sites Liptovská Teplička and Tajov. Biotic (microbial community structure, earthworm number and fresh body biomass, arthropod number and fresh body biomass), and abiotic chemical soil parameters (pH, total organic carbon, total nitrogen, nutrients) were measured. According to MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight), several bacterial strains were identified. Mutual relations between soil microorganisms and soil biotic and abiotic properties determined by different land uses were analysed. Microbial response expressed as average well-colour development (AWCD) values indicated relations between higher microbial diversity and higher nutrient availability at both study sites. In the comparison of land use types, permanent grasslands (PG) showed the lowest microbial activity in the depth of 0–0.1 m. But in the depth of 0.2-0.3 m in PG of both study sites, the higher microbial activity was recorded compared to the depth of 0-0.1 m. In addition, lower AWCD values in PG were in line with the lower available P and K content but higher earthworm density and biomass.

Key words: biotic soil properties, chemical soil properties, MALDI, arable land, permanent grasslands, abandoned grasslands, forest land.

Introduction

Soils are heterogenous mixtures of mineral, organic and biological compounds, which are frequently associated in complex hierarchical structures. Microbes adapt to microhabitats and live together in consortia interacting with each other and their environment. Microbial diversity is critical to ecosystem functioning due to diversity of process, such as decomposition, nutrient cycling, soil aggregation and pathogenicity (Dubey et al., 2006) According to a current estimate, 1 g of soil may harbour up to 10 billion bacteria of possibly 4000–7000 different species and a biomass density of 30–30,000 $kg.ha^{-1}$ (Rosello-Mora, Amann, 2001).

The microbial members of soil communities are the most sensitive and rapid indicators of perturbations and land use changes. Land use change is a key component of global changes and largely impacts ecosystem structures, processes and functioning (Don et al., 2011). Soil biota contributes substantially to the resistance and resilience of agroecosystems to abiotic disturbance and stress (Brussaard et al., 2007). But the excessive use of pesticides can drastically modify the function and structure of soil microbial communities (Pampulha, Oliveira, 2006). Soils subjected to disturbance by tillage, however, can be more susceptible to reductions in soil microbiota due to desiccation, mechanical destruction, soil compaction, reduce pore volume, and disruption of access to food resources (Giller, 1996).

Increase in land use intensity, due to the demands of bioeconomy (production of food, feed, fuel and fibre), is also frequently observed in grassland ecosystems. While in the past, sites have been used extensively as pastures, nowadays, up to four times per season, the same areas are managed as meadows for hay production and silage, entailing an intensive application of organic and inorganic fertilizers (Meyer et al., 2013). Traditional agricultural landscape consists of a mosaic of small-scale arable fields and permanent agricultural cultivation such as grasslands. They are significant as unique islands of species-rich plant and animal communities (Barančoková, Barančok, 2015). Differences in intensity of agricultural practice like mowing, grazing and fertilization lead to changes in land use, plant composition (Kaschuk et al., 2009, Thurston et al., 1969, Kirkham et al., 1996), microclimate, soil quality, and hence, to changes on macro- as well as micro-scale habitats. For example, Patra et al. (2006) compared the diversity pattern of microbial community involved in nitrogen fixation, denitrification and nitrification in grassland ecosystems under different management intensities. This study clearly demonstrated the changes in the diversity pattern of single functional groups involved in nitrogen transformation on low diverse grassland sites.

Soil microorganisms, mainly fungi and bacteria, are primarily responsible for the transformation of organic molecules in soil, and their activity is thus a key factor in SOM dynamics (Coq et al., 2007). Soil microorganisms are the decomposers of litter and SOM (soil organic matter) in terrestrial ecosystems, which can regulate multiple input and loss pathways of soil C and nitrogen (N) (McGuire, Treseder, 2010, Smith et al., 2014). It has been suggested that land use change can affect the microbial decomposition of litter and SOM, which in turn regulates soil C and N balance in terrestrial ecosystems (Brackin et al., 2013, Bossio et al., 2006).

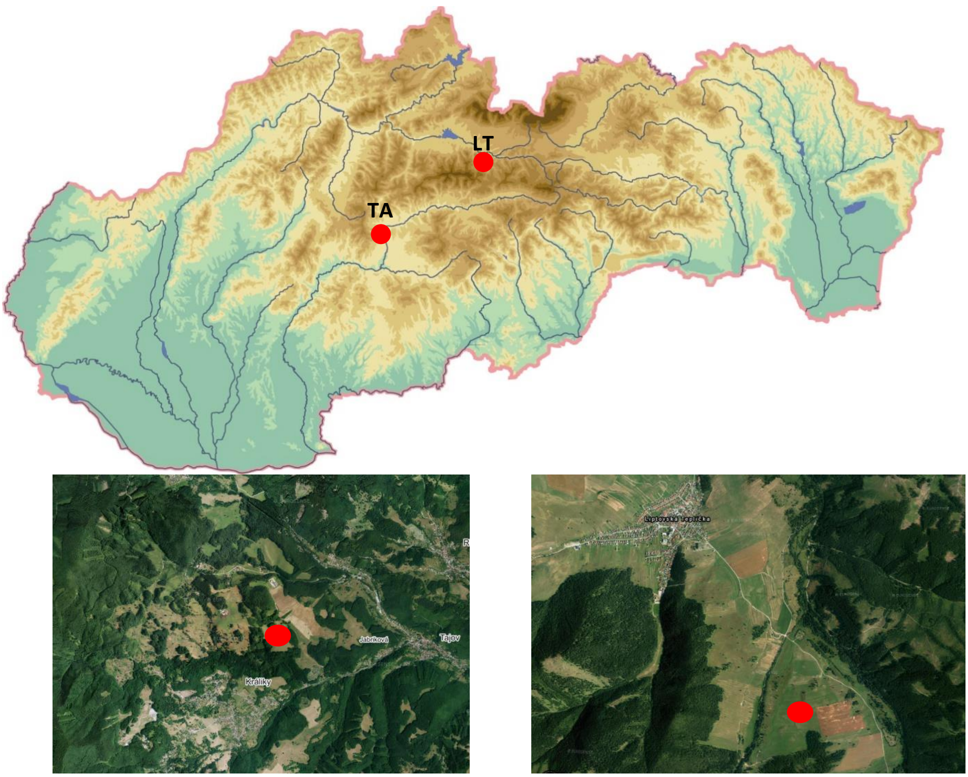
Soil biota has considerable direct and indirect effects on crop growth and quality, nutrient cycle quality and the sustainability of soil productivity (Roger-Estrade et al., 2010). The researchers evaluate not only the roles of total carbon and nitrogen on microbial diversity but also the role of other nutrients, their availabilities and ratios, together with land use, climate and biotic and abiotic factors. Delgado-Baquerizo et al. (2016) found that bacterial diversity and composition is primarily driven by variation in soil resources stoichiometry (total C:N:P ratios), itself linked to different land uses, and secondarily driven by other important biodiversity drivers such as soil pH, root influence and so on.

The study reports the microbial activity and diversity under different land use types in the year 2015 (partially in 2014) in the Slovak mountain regions with the following objectives: (1) to determine microbial activity and diversity in two different soil types and four land use types; (2) to determine selected soil chemical properties in different soil types and land use types (3) to evaluate mutual relations between soil biotic and abiotic parameters.

Material and methods

Study site characteristics

Slovakia is predominantly situated in the western Carpathian arch. Nowadays, agricultural land in Slovakia occupies almost 50% of the total area of Slovakia. But during the formation period of Neolithic agriculture, the Carpathians were almost completely covered with forests (Ložek, 1973). It means that the landscape of Slovakia has undergone significant changes in land-use and cover until now. The study was carried out in 2014–2015 on two study sites,



TA – Tajov LT – Liptovská Teplička

Fig. 1. Map of the location of two study sites in Slovakia (TA – Tajov, LT – Liptovská Teplička).

Rendzic Leptosol (FAO, 2014) developed on limestones in the region of north-eastern Slovakia at Liptovská Teplička (LT), and Haplic Cambisol (dystric variety) (FAO, 2014) developed on slope sediments in the region of central Slovakia at Tajov (TA). The LT area is situated in the Low Tatras Mountain at 950 m a.s.l. The long-term average annual air temperature is 6.2 °C, and long-term average annual rainfall is 950 mm. From the long-term point of view, the main land-use trend observed in LT was gradual afforestation and permanent grassland conversion to forest land (Kanianska et al., 2014). At present, extensive land abandonment in mountain rural areas continues. The TA area is situated in the Kremnica Mountain at 595 m a.s.l. The long-term average annual air temperature is 7.2 °C, and long-term average annual rainfall is 795 mm. The landscape suffers many changes that reflects in soil properties and mutual interactions between biotic and abiotic parameters. Therefore, we observed biotic with emphasis on soil microorganisms and abiotic properties and their mutual interactions in four different land uses in LT (arable land – AL, permanent grasslands – PG, abandoned grasslands – AG, forest land – FL), and in two different land uses in TA (permanent grasslands – PG, forest land – FL). In LT, the arable land is under organic farming, and the permanent grasslands are used as a meadow, and are mown for hay. In TA, the permanent grasslands are grazed by sheep.

Biotic soil properties methods

The investigation was conducted in the autumn season in 2015; the selected analyses was conducted in the autumn and spring seasons in 2014. From the biotic soil properties, the microbial communities and their diversity were investigated.

Soil samples for microbial analyses were collected from 0–0.1 m and 0.20–0.30 m depth from four points placed at the apices of a 10 m side square at both study sites (LT, TA), and in the available land-use types (AL, PG, AG, FL). Soil samples from autumn 2015 were processed for community level physiological profiling (CLPP) using Biolog EcoPlates. Selected colonies from the samples of autumn 2015 and also spring and autumn 2014 were analysed according to Matrix Assisted Laser Desorption Ionization-Time of Flight Bruker Daltonik MALDI Biotyper, MicroflexLT (MALDI-TOF).

Microorganism analysis - The Biolog EcoPlates

Microbial communities offer a potentially powerful forum for advancing the understanding on how community processes affect ecosystem processes. Also, active organisms, not culturable using solid agar, can respond in the CLPP. Organisms adapted for high nutrient concentration and rapid growth (like Pseudomonads) responds well on this assay (Garland, 1997). The Biolog EcoPlate (Garland, 1997) contains 31 of the most useful carbon sources for soil community analysis. Communities of organisms gave a characteristic reaction pattern called a metabolic fingerprint. Catabolism of each carbon substrate produced a proportional colour change response (from the colour of the inoculant to dark purple) due to the activity of the redox dye tetrazolium violet (present in all wells including blanks). The homogenized soil sample (1 g) was incubated in 99 ml of sterile water for 20 min. at 20 °C. Environmental samples' (150 μl) suspensions (soil) were then inoculated directly into Biolog MicroPlates (Frac et al., 2012). The Biolog MicroPlates were incubated in the dark at 25 °C and analysed at defined time intervals over 2–5 days using microplate reader by absorbance at 590 nm (Technical University, Zvolen, Slovakia).

The rate of utilization was indicated by the reduction of the tetrazolium, a redox indicator dye that changes from colourless into purple. The changes in the pattern were compared via the Principle Components Analysis (PCA) of the average well colour development (AWCD) data (Firestone et al., 1997; Cartwright, 2015). Microbial response in each microplate that expressed average well-colour development (AWCD) was determined as follows AWCD = Σ ODi/31 (Gomez et al., 2004), where ODi is optical density value from each well, corrected subtracting the blank well (inoculated, but without a carbon source) values from each plate well. The changes observed in the fingerprint pattern provide useful data about the microbial population changes over time, and generate a microbial community summary variable based on substrate utilization.

Microorganism analysis - Cultivation and Matrix assisted laser desorption/ionization, time of flight – MALDI-TOF

Selected microbial colonies were analysed according to MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight Bruker Daltonik MALDI Biotyper, MicroflexLT, Matej Bel University, Banska Bystrica, Slovakia). Preparation of soil samples' suspensions: 0.5 g of soil was homogenized in 2 ml of sterile phosphate buffer (PBS). Water and soil suspension samples /50–100 μl/ were placed on TSA (Trypton Soya agar, Oxoid) medium and cultivated for 24 hours by 35 °C, and then, the concentration of colonies, colony forming units (cfu) were done. Selected colonies were analysed according to the standard microbial protocols and according to the dry and semi-extraction procedure of MALDI–TOF (Matrix assisted laser desorption/ionization, time of flight) method (Bruker protocols).

Soil chemical and physical analyses

The investigation was conducted in the autumn season in 2015. On each land-use type, soil samples for chemical analysis were collected from the same places like samples for microbial analyses, from four points placed at the apices of a 10-m side square. Soil samples were air dried, homogenized, sieved on 2 mm sieves and analysed according to the methods applied in Partial monitoring system – Soil (Fiala, 1999). Soil pH was determined in KCl, total oxidizable organic carbon (TOC) according to Tjurin´s method in the modification of Nikitin, total nitrogen (N_t) according to the modified Kjeldahl method in accordance with the Slovak standardized method (STN ISO 11261), available nutrients (P, K, Mg) according to Mehlich III. The average values were used as soil chemical status characteristics. Content of clay was determined by the pipette method according to Novak.

Earthworm and arthropod sampling and determination

The investigation was conducted in the spring season in 2015. We used the obtained results for the evaluation of the possible mutual relations between microorganisms and soil meso (soil arthropods) and macrofauna (earthworms).

Earthworms and soil arthropods were sampled in each land-use type. At each site, earthworms were hand sorted from seven soil monoliths (0.35 x 0.35 x 0.20 m) placed in line in 3 m distance. The earthworms were counted and collected. Earthworms from deeper soil layers were expelled by 1.5 L of 0.2% formalin. The collected samples of earthworms were transported in glass cups with sufficient amount of soil in portable fridge to laboratory. The collected earthworms were washed, weighted and the body colour was noted. The earthworm density and biomass from soil monoliths were recalculated per square meter (Kanianska et al., 2016).

Soil arthropods were sampled from the same places where earthworms were collected into the 7 plastic traps placed flush with the surface of the soil in line in 3 m distance. The traps were filled with 200 ml formalin solution, which acted as a killing and preserving agent. After one month, the traps were collected and the material was weighed. The captured individuals were preserved in formalin solution, identified, and the total number of each one was recorded and classified in taxonomic categories (orders) (Jaďuďová et al., 2016). Quantitative composition was expressed as number of soil arthropod individuals ($ind.trap^{-1}$) and fresh body biomass ($g.trap^{1}$).

Results

Microbial community structure

Measurements of metabolic traits through different carbon sources utilization were used like an indicator of biotic parameters in different soil communities. In the abandoned grasslands (AG) and forest land (FL) at Liptovská Teplička (LT) and in the forest land (FL) at Tajov (TA), more colour intensity differences were obtained than in the permanent grasslands (PG) and arable land (AL) with a lot of colourless results, especially at TA (Fig. 2).

We compared the average well-colour development data at the two study sites and different land-uses (Tables 6, 7 and 8). At LT, based on the AWCD data we can assume in the depth of 0–0.1 m, the highest microbial diversity in AG (0.820) followed by AL (0.685) and FL (0.698). The lowest microbial diversity was found in PG (0.290). Similarly at TA, the higher microbial diversity based on AWCD data can be assumed in FL (0.801) as compared to PG (0.286). In the depth of 0.2–0.3 m in PG at both study sites, the microbial activity was higher as compared to the depth of 0–0.1 m but still the lowest as compared to the other land uses. The microbial activity at LT in AL was in the depth of 0.2–0.3 m lower (0.433) than in the depth of 0–0.1 m (0.685); in AG, the situation was opposite and the microbial activity was slightly higher in the depth of 0.2–0.3 m (0.877) than in the depth of 0–0.1 m (0.820).

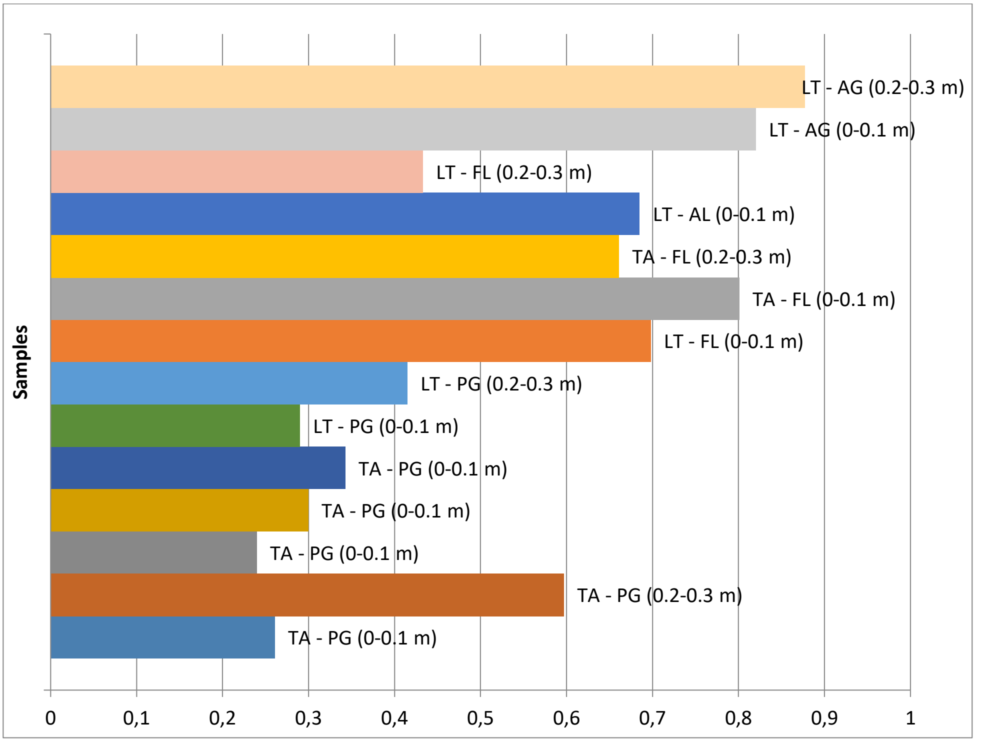


Fig. 2. Average well-colour development (AWCD) comparison of samples.
Notes: LT – Liptovská Teplička, TA – Tajov, AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

Microbial community diversity

Selected microbial colonies after cultivation of TSA (Trypton Soya Agar) were analysed according to MALDI-TOF at LT and TA study sites. Several bacteria strains were identified in different land uses and in different time period (Tables 1– 5).

In autumn samples from 2014 and 2015, several bacterial strains were obtained that were more variable in comparison with the spring samples strains, probably because of low temperature in the environment and slow start of biological processes in the soil.

There were found different strains of microorganisms like *Acinobacter calcoaceticus, Stenophomonas rhizophila, Solibacilus silvestris, Pseudomonas fluorescens, P. frederiksbergensis* and *Serratia proteamaculans.* At LT, the most frequent microorganisms in autumn 2015

T a b l e 1. MALDI-TOF results at Liptovská Teplička in autumn 2015.

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
(++) (A)	AL1 (0–0.1 m)	*Pseudomonas frederiksbergensis*	2.117	*Pseudomonas brassicacearum*	1.916
(+) (B)	AL1 (0.2–0.3 m)	*Stenotrophomonas rhizophila*	1.867	*not reliable identification*	1.434
(+) (B)	AL2 (0.2–0.3 m)	*Acinetobacter calcoaceticus*	1.895	*not reliable identification*	1.678
(+) (B)	PG1 (0–0.1 m)	*Pseudomonas caricapapayae*	1.79	*not reliable identification*	1.598
(+) (B)	PG1 (0.2–0.3 m)	*Stenotrophomonas sp*	1.992	*not reliable identification*	1.362
(+) (B)	PG2 (0.2–0.3 m)	*Acinetobacter calcoaceticus*	1.847	*Acinetobacter calcoaceticus*	1.821
(+) (B)	PG3 (0.2–0.3 m)	*Acinetobacter calcoaceticus*	1.872	*Acinetobacter calcoaceticus*	1.869
(++) (A)	AG1 (0–0.1 m)	*Acinetobacter calcoaceticus*	2.268	*Acinetobacter calcoaceticus*	2.182
(++) (A)	AG2 (0–0.1 m)	*Acinetobacter calcoaceticus*	2.104	*Acinetobacter calcoaceticus*	2.036
(++) (A)	AG1 (0.2–0.3 m)	*Acinetobacter calcoaceticus*	2.253	*Acinetobacter calcoaceticus*	2.206
(+++) (A)	AG2 (0.2–0.3 m)	*Acinetobacter calcoaceticus*	2.329	*Acinetobacter calcoaceticus*	2.327
(++) (A)	AG3 (0.2–0.3 m)	*Acinetobacter calcoaceticus*	2.016	*Acinetobacter calcoaceticus*	1.987
(+) (B)	FL1 (0–0.1 m)	*Serratia proteamaculans*	1.897	*Serratia liquefaciens*	1.885
(+) (B)	FL2 (0–0.1 m)	*Stenotrophomonas rhizophila*	1.713	*not reliable identification*	1.365

Notes: AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

T a b l e 2. MALDI-TOF results at Liptovská Teplička in autumn 2014.

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
(+++)(A)	AG1 (0–0.1 m)	*Serratia fonticola*	2.318	*Serratia fonticola*	2.086
(++) (B)	AG2 (0–0.1 m)	*Serratia quinivorans*	2.14	*Serratia proteamaculans*	2.013
(++) (A)	AG3 (0–0.1 m)	*Serratia plymuthica*	2.149	*Serratia plymuthica*	2.122
(+++) (A)	AG1 (0.2–0.3 m)	*Serratia plymuthica*	2.31	*Serratia plymuthica*	2.231
(++) (A)	AG2 (0.2–0.3 m)	*Solibacillus silvestris*	2.103	*not reliable identification*	1.468
(++) (A)	AG3 (0.2–0.3 m)	*Myroides odoratus*	2.297	*Myroides odoratus*	2.281
(+) (B)	FL1 (0–0.1 m)	*Bacillus pumilus*	1.905	*Bacillus pumilus*	1.89
(++) (B)	FL2 (0–0.1 m)	*Viridibacillus neidei*	2.247	*Viridibacillus arvi*	2.02
(++) (A)	FL3 (0–0.1 m)	*Serratia quinivorans*	2.168	*Serratia proteamaculans*	1.999
(+) (B)	FL4 (0–0.1 m)	*Solibacillus silvestris*	1.951	*not reliable identification*	1.513
(++) (A)	FL5 (0–0.1 m)	*Serratia plymuthica*	2.197	*Serratia plymuthica*	2.152
(+) (B)	FL6 (0–0.1 m)	*Viridibacillus neidei*	1.872	*not reliable identification*	1.67
(+) (B)	FL7 (0–0.1 m)	*Serratia quinivorans*	1.854	*Serratia liquefaciens*	1.747

Notes: AG – abounded grasslands, FL – forest land.

was *Acinetobacter calcoaceticus* that was probably determined by calcareous substrate (dolomitic limestone). The genus *Acinetobacter* is defined as including gram-negative coccobacilli that are strictly aerobic with a high ability to utilize phenol as the sole source of carbon and energy (Bergogne-Bérézin, Towner, 1996). Thus, phenolic compounds can be degraded by this microbe, and thus, remediate phenol contaminated soil. Most *Acinoteobacter* strains can grow in a simple mineral medium (Cordova-Rosa et al., 2009).

T a b l e 3. MALDI-TOF results at Tajov in autumn 2015.

Analyte Name	**Analyte ID**	**Organism (best match)**	**Score Value**	**Organism (second best match)**	**Score Value**
(+) (B)	PG1 (0–0.1 m)	*Bacillus cereus*	1.846	*Bacillus cereus*	1.824
(+) (B)	FL1 (0–0.1 m)	*Bacillus thuringiensis*	1.976	*Bacillus cereus*	1.751
(+) (B)	FL1 (0.2–0.3 m)	*Bacillus weihenstephanensis*	1.951	*Bacillus thuringiensis*	1.919
(+) (B)	FL2 (0–0.1 m)	*Bacillus thuringiensis*	1.936	*Bacillus cereus*	1.903
(+) (B)	FL2 (0.2–0.3 m)	*Bacillus cereus*	1.97	*Bacillus thuringiensis*	1.947

Notes: PG – permanent grasslands, FL – forest land.

T a b l e 4. MALDI-TOF results at Tajov in spring 2015.

Analyte Name	**Analyte ID**	**Organism (best match)**	**Score Value**	**Organism (second best match)**	**Score Value**
(+) (B)	PG1 (0–0.1 m)	*Pseudomonas chlororaphis*	1.979	*Pseudomonas corrugata*	1.964
(++) (B)	PG2 (0–0.1 m)	*Pseudomonas libanensis*	2.159	*Pseudomonas fluorescens*	2.081
(+) (B)	PG3 (0–0.1 m)	*Pseudomonas brassicacearum*	1.847	*Pseudomonas kilonensis*	1.804
(++) (A)	PG4 (0–0.1 m)	*Pseudomonas fluorescens*	2.056	*Pseudomonas corrugata*	1.87
(+) (B)	PG5 (0–0.1 m)	*Pseudomonas extremorientalis*	1.874	*Pseudomonas chlororaphis*	1.837
(+) (B)	PG1 (0.2–0.3 m)	*Pseudomonas chlororaphis*	1.815	*Pseudomonas koreensis*	1.804

Notes: PG – permanent grasslands, FL – forest land.

T a b l e 5. MALDI-TOF results at Tajov in autumn 2014.

Analyte Name	**Analyte ID**	**Organism (best match)**	**Score Value**	**Organism (second best match)**	**Score Value**
(+) (B)	PG1 (0–0.1 m)	*Bacillus cereus*	1.846	*Bacillus cereus*	1.824
(+) (B)	FL1 (0–0.1 m)	*Bacillus thuringiensis*	1.976	*Bacillus cereus*	1.751
(+) (B)	FL2 (0–0.1 m)	*Bacillus weihenstephanensis*	1.951	*Bacillus thuringiensis*	1.919
(+) (B)	FL3 (0–0.1 m)	*Bacillus thuringiensis*	1.936	*Bacillus cereus*	1.903
(+) (B)	FL4 (0–0.1 m)	*Bacillus cereus*	1.97	*Bacillus thuringiensis*	1.947

Notes: PG – permanent grasslands, FL – forest land.

Explanatory Notes:

Range	**Description**	**Symbols**
2.300 ... 3.000	Highly probable species identification	(+++)
2.000 ... 2.299	Secure genus identification, probable species identification	(++)
1.700 ... 1.999	Probable genus identification	(+)
0.000 ... 1.699	Not reliable identification	(-)

Meaning of Consistency Categories (A - C):

Category	**Description**
A	**Species Consistency:** The best match was classified as "green" (see above). Further "green" matches are of the same species as the first one. Further "yellow" matches are at least of the same genus as the first one.
B	**Genus Consistency:** The best match was classified as "green" or "yellow" (see above). Further "green" or "yellow" matches have at least the same genus as the first one. The conditions of species consistency are not fulfilled.
C	**No Consistency:** Neither species nor genus consistency. (Please check for synonyms of names or microbial mixture.)

Stenotrophomonas species have an important ecological role in the element cycle in nature (Ikemoto et al., 1980). Growth takes place at 4–37 °C. They are resistant to many antibiotics, for example, penicillin, tobramycin, imipenem and ceftazidime but susceptible to chloramphenicol, kanamycin and trimethoprim, sulfamethoxazole. Strains were plant-associated and isolated from the rhizosphere of oilseed rape and from the rhizosphere and geocaulsophere (tuber) of potato. Endophytic colonization was found (Wolf et al., 2002). Colonies of *Stenotrophomonas rhizophila* are yellowish. The strain uses xylose as a carob source. Antagonistic activity was shown against plant-pathogenic fungi. They are not active against bacteria (Wolf et al., 2002).

A novel estuarine bacterial strain, *Solibacillus silvestris* AM1, produces an extracellular, thermostable and fibrous, glycoprotein bioemulsifier (BE-AM1). Cell-bound BE-AM1 production by *S. silvestris* AM1 during the mid-logarithmic phase of growth coincided with a decrease in cell surface hydrophobicity, and an increase in cell autoaggregation and biofilm formation. Markande and Nerurkar (2016) study has revealed that the BE-AM1, a bacterial bioemulsifier, is a functional amyloid and has a role in biofilm formation and cell surface modulation in *S. silvestris* AM1.

Pseudomonas species are important decomposers of organic matter in soil, but are also pathogens in plants, animals and humans (Palleroni, 1993). The genus *Pseudomonas* was previously a heterogenous group of species, defined by a limited number of phenotypic characters. At present, the genus based on phylogenetic rRNA homology studies, several groups formerly belonging to Pseudomonas have been reclassified (Kersters et al., 1996). During the past decade, several new Pseudomonas species have been described. *Pseudomonas fluorescens* is an obligate aerobe, with an extremely versatile metabolism, and can be found in the soil and in water, is non-pathogenic (Frank, 1997). In common, the most of in spring-soils samples obtained strains were endospore forming, psychrotolerant species, like *Pseudomonas*, which correspond with climate, seasonal and geographical conditions, low temperature in the environment. *P. frederiksbergensis* pertaining to Frederiksberg near Copenhagen, Denmark, from where the organism was isolated. Cells are gram-negative, motile, non-spore-forming rods. Colonies are smooth and pale yellowish on agar. The species is oxidase- and catalase-positive, denitrifying, and shows hydrolysis of gelatin and accumulation of PHB. The species shows no acidification of glucose or hydrolysis of starch (Andersen et al., 2000). *Serratia proteamaculans* improve plant growth and/or nitrogen fixation in legume plants.

Soil chemical properties

The differences in microbial diversity between both study sites and different land uses are conditioned besides land management also by natural factors including soil chemical parameters. Clay content is higher at TA (33.78% in AL) than at LT (26.72% in AL). The status of the AWCD and selected soil chemical parameters are listed in Tables 6 and 7 for LT and TA, respectively.

Rendzic Leptosol at LT is developed on dolomitic limestones that reflects in neutral pH values ranging from 6.86 in PG (depth 0–0.1 m) to 7.02 in FL (depth 0–0.1 m). The amount of organic carbon and nutrient varied. The higher content of organic carbon and nutrients

T a b l e 6. AWCD data and soil chemical parameters (average ± standard deviation) at Liptovská Teplička.

Depth (m)	Land use	AWCD	pH KCl	TOC ($g \cdot kg^{-1}$)	N_t ($g \cdot kg^{-1}$)	P ($mg \cdot kg^{-1}$)	K ($mg \cdot kg^{-1}$)	Mg ($mg \cdot kg^{-1}$)
0–0.1	AL	0.685	6.76 ± 0.14	27.70 ± 8.60	6.19 ± 0.64	30.25 ± 3.71	110.66 ± 15.71	931.63 ± 62.18
	PG	0.290	6.86 ± 0.22	55.40 ± 8.59	9.33 ± 0.83	3.58 ± 0.54	99.61 ± 7.06	1322.92 ± 99.05
	AG	0.820	6.99 ± 0.35	47.00 ± 7.94	6.85 ± 0.79	54.53 ± 11.33	124.85 ± 14.73	1049.49 ± 110.06
	FL	0.698	7.02 ± 0.32	63.80 ± 4.49	9.63 ± 1.69	9.35 ± 3.70	99.61 ± 12.01	1325.22 ± 140.50
0.2–0.3	AL	0.433	6.99 ± 0.16	21.20 ± 6.70	3.81 ± 0.62	25.32 ± 4.24	80.21 ± 6.70	737.85 ± 94.54
	PG	0.415	6.87 ± 0.21	39.20 ± 7.31	7.08 ± 0.67	2.66 ± 0.40	80.21 ± 8.14	1144.25 ± 157.20
	AG	0.877	6.97 ± 0.21	23.20 ± 4.93	3.99 ± 1.27	45.93 ± 3.81	67.81 ± 9.78	708.81 ± 56.28

Notes: AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

T a b l e 7. AWCD data and soil chemical parameters (average ± standard deviation) at Tajov.

Depth (m)	Land use	AWCD	pH KCl	TOC ($g \cdot kg^{-1}$)	N_t ($g \cdot kg^{-1}$)	P ($mg \cdot kg^{-1}$)	K ($mg \cdot kg^{-1}$)	Mg ($mg \cdot kg^{-1}$)
0–0.1	PG	0.286	4.98 ± 0.40	41.10 ± 7.93	7.59 ± 0.57	1.85 ± 0.19	104.72 ± 13.77	755.66 ± 429.04
	FL	0.801	5.06 ± 0.46	145.44 ± 11.33	7.38 ± 0.49	13.49 ± 2.43	329.74 ± 36.23	717.57 ± 130.78
0.2–0.3	PG	0.597	4.85 ± 0.42	20.65 ± 6.83	4.39 ± 0.62	0.86 ± 0.23	102.24 ± 19.01	782.73 ± 521.01
	FL	0.661	3.72 ± 0.11	32.42 ± 6.57	2.16 ± 0.47	1.25 ± 0.38	139.54 ± 16.38	700.32 ± 74.69

Notes: PG – permanent grasslands, FL – forest land.

are in the depth of 0–0.1 m compared to the depth of 0.2–0.3 m. The highest total organic carbon content in the depth of 0–0.1 m is in FL (63.80 $g \cdot kg^{-1}$) with the second highest AWCD values. The highest AWCD value was recorded in AG with lower TOC content but higher available phosphorus and potassium content. Because of the dolomitic substrate, the soil is rich in magnesium. Overall, the content of P and K are relatively low according to the evaluation of nutrient content in soil realized by the Central Control and Testing Institute in Agriculture in frame of agrochemical testing of soil (CCTIA, 2013).

Soil reaction in Haplic Cambisol, dystric variety, at TA ranged from 3.72 in FL (depth 0.2–0.3 m) that means extremely acid soil reaction to 5.06 in FL (depth 0–0.1 m) what is strong acid soil reaction. The higher content of organic carbon and nutrients are in the depth of 0–0.1 m compared to the depth of 0.2–0.3 m. The amount of organic carbon is substantially higher in FL compared to PG. Similarly, the AWCD values are higher in FL compared to PG. The soil in FL and PG is rich in magnesium, and poor in phosphorous. Potassium content is low in PG according to the evaluation of nutrient content in soil realized by the Central Control and Testing Institute in Agriculture in frame of agrochemical testing of soil (CCTIA, 2013).

Soil biotic properties

The differences in microbial diversity can be affected by other living organisms in soil. We observed the numbers and fresh body biomass of earthworms and soil arthropods at LT and TA in spring 2015 (Table 8) (Kanianska et al., 2016) to observe the possible interactions with soil microorganisms.

T a b l e 8. AWCD data and averaged soil biotic parameters at Liptovská Teplička and Tajov.

Study site	Land use	AWCD	Earthworm biomass ($g \cdot m^{-2}$)	Earthworm density ($ind.m^{-2}$)	Arthropod biomass ($g \cdot trap^{-1}$)	Arthropod density ($ind.trap^{-1}$)
LT	AL	0.685	7.23	20.99	13.84	32.71
	PG	0.290	28.20	74.60	3.08	23.29
	AG	0.820	5.10	9.00	3.23	35.28
	FL	0.698	0.70	3.30	0.82	6.42
TA	PG	0.433	40.80	108.50	1.53	31.43
	FL	0.415	9.40	18.70	2.85	21.86

Notes: LT – Liptovská Teplička, TA – Tajov, AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

At both study sites, the highest earthworm density and body biomass within different land uses were observed in PG. In LT, the highest arthropod biomass and density were observed in AL. In TA, the higher arthropod biomass was in FL but the arthropod density was higher in PG.

Discussion

Microbial activity and land management

Land management reflected in the microbial activity. In AL at LT, we observed relatively high microbial activity. The area is under organic farming that assumes sustainable land management.

Agricultural practices have proven to be unsuitable in many cases, causing considerable reductions in soil quality. Land management practices can provide solutions to this problem and contribute to get a sustainable agriculture model. García-Orenes et al. (2013) studied the effect of different agricultural management practices on soil microbial community structure. Their results showed a substantial level of differentiation in the microbial community structure, in terms of management practices, which was highly associated with soil organic matter content. The microbial community composition of the abandoned agricultural soil was characterised by increases in both fungal abundances and the metabolic quotient, suggesting an increase in the stability of organic carbon. The ratio of bacteria:fungi was higher in wild forest coverage and land abandoned systems, as well as in the soil treated with oat straw. Similarly, in our study, the abandoned grasslands and forest land were typical in higher microbial activity than the managed permanent grasslands mown for hay.

Microbial activity and soil chemical properties

Microbial activity is affected by various abiotic factors. Prokaryotic communities in soils are among the most taxon rich of any microbial habitat (Madigan, 2008), and the abiotic heterogeneity in soils is a major contributor to their biological diversity (Fierer, Lennon, 2011).

Salinity and pH are the major drivers of microbial communities in all habitats and, especially, in soils (Herlemann, 2011). It is expected that agricultural land use change may impact soil microbial community composition and biomass, primarily by soil properties such as pH, soil depth, moisture and temperature (Lauber et al., 2009). In our research, at LT study site, the highest microbial activities were observed in AG followed by FL with higher pH values than in the other two land-uses (Table 6). At the TA study site, the higher pH value was observed in FL with higher microbial activity than in PG (Table 7).

The distribution of bacteria also depends on the content of organic carbon (OC) and clay (Ranjard et al., 2000). The surfaces of micro and macro aggregates differ in their physico-chemical properties and provide habitats for soil microorganisms (Ditterich et al., 2016), frequently attached to mineral surfaces or organo-mineral complexes. Ditterich et al. (2016) showed that changes in substance availability as well as mineral properties are important drivers for the development of microbial communities. The ability of a few soil bacteria to transform unavailable forms of phosphorus (P) and potassium (K) to an available form is an important feature in plant growth (Jat et al., 2015; Kumar et al., 2016). In our research, higher AWCD values indicate relations between higher microbial diversity and higher nutrient availability.

Soil (represented by AL) at the TA study site has higher content of clay as compared to the soil at LT. FL at the TA study site also has the highest total organic carbon content within all land-uses (Table 6). High AWCD data (0.801) indicate high microbial activity that can be determined by these two factors. On the other hand, in the samples of Klin and Mutne peatlands /Slovakia/ high share of genera *Bacillus flexus*, *Serratia liquefaciens, Pseudomonas proteolytica*, *P. fragi*, *P. chlororaphis* were identified (Júdová et al., 2015), similar to the permanent grasslands and forest lands at Tajov (Tables 3, 4). Klin peatland is bog-forest type and Mutne is active raised bogs with a mosaic of alkaline fens, threatened by succession and decreasing underground water level.

Besides the abiotic factors, plants exert strong controls on the composition of bacterial communities in vegetated soils (Zak et al., 2003). Also, our results showing differences in AWCD data (Tables 6, 7) can be determined not only by chemical and physical soil parameters but also by plant composition as a consequence of different land use types. There is also a possibility that within one land use type, the effect of different plants is recorded. Urbanová et al. (2015) have reported that the effects of the tree species in a forest ecosystem explain a large proportion of variation in microbial community composition than other soil properties, especially in fungi.

Microbial activity and soil biotic parameters

Microbial activity is affected by the activity of other groups of organisms living in soil. The biochemical decomposition of OM (organic matter) is primarily accomplished by microorganisms, but earthworms are crucial drivers of the process, as they may affect microbial decomposer activity by grazing directly on microorganisms (Aira et al., 2009), and by increasing the surface area available for microbial attack after comminution of OM (Domínguez et al., 2010). Some microorganisms may be a source of food for earthworms, but the amounts consumed and the ability of earthworms to digest and assimilate microbial biomass vary with earthworm species, its ecological category, food substrate and the environmental conditions in which the earthworms are living (Brown, Doube, 2004). In our study, we found out such possible effects of earthworms on microorganisms. Figures 3 and 4 show mutual interactions between soil activity expressed by AWCD values and earthworm density and biomass. The higher microbial activity is connected with the lower earthworm density and biomass in the depth 0–0.1 m.

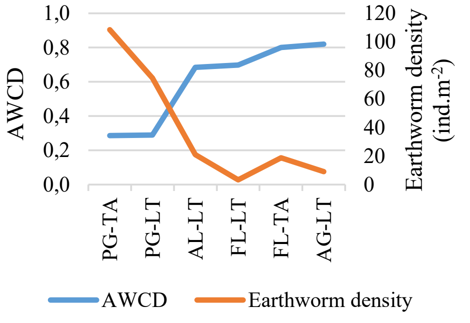


Fig. 3. Average well-colour development (AWCD) and earthworm density at Liptovská Teplička and Tajov. Notes: LT – Liptovská Teplička, TA – Tajov, AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

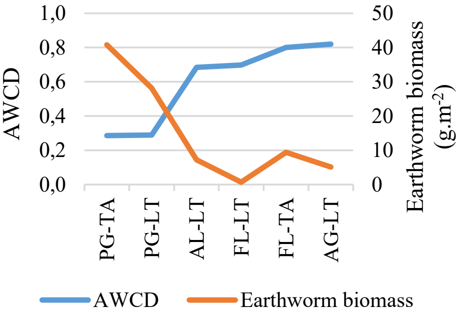


Fig. 4. Average well-colour development (AWCD) and earthworm biomass at Liptovská Teplička and Tajov. Notes: LT – Liptovská Teplička, TA – Tajov, AL – arable land, PG – permanent grasslands, AG – abounded grasslands, FL – forest land.

Conclusion

At both study sites, we found the differences in the microbial activity conditioned by the different land use. There were found different strain of microorganisms like *Acinobacter calcoaceticus, Stenophomonas rhizophila, Solibacilus silvestris, Pseudomonas fluorescens, P. frederiksbergensis, Seratia proteamaculans.* The highest microbial activity deduced on AWCD values in the depth of 0–0.1 m was observed at the TA study site in FL with higher TOC, available P and K content. At the LT study site, the highest microbial activity was recorded in AG followed by FL and organically managed AL. Higher AWCD values indicate relations between higher microbial diversity and higher nutrient availability at both study sites. In the comparison of land use types, permanent grasslands showed the lowest microbial activity in the depth of 0–0.1 m. But in the depth of 0.2–0.3 m in PG of both study sites, the higher microbial activity was recorded as compared to the depth of 0–0.1 m. In addition, lower AWCD values in PG were in line with the lower available P and K content but higher earthworm density and biomass.

Acknowledgements

This work was supported by the Slovak Research and Development Agency for the financial support under Grant No. APVV-0098-12 *Analysis, modelling and evaluation of agroecosystem services* and VEGA 1/0589/17 *The modification of advanced materials and composites by physical and chemical methods*. The research of abiotic soil parameters and MALDI analyses were done by the equipment supported by Operational Programme Research and Development via contract No. ITMS- 26210120024 *Restoration and building of infrastructure for ecological and environmental research at Matej Bel University in Banská Bystrica*.

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Ekológia (Bratislava) Vol. 38, No. 2, p. 117–125, 2019
DOI:10.2478/eko-2019-0009

CYTOGENETIC ABNORMALITIES IN SEED PROGENIES OF *Pinus pallasiana* D. DON STANDS FROM TECHNOGENIC POLLUTED LANDS IN THE STEPPE OF UKRAINE

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Abstract

Korshikov I., Belonozhko Y., Lapteva H.: Cytogenetic abnormalities in seed progenies of *Pinus pallasiana* D. Don stands from technogenic polluted lands in the steppe of Ukraine. Ekológia (Bratislava), Vol. 38, No. 2, p. 117–125, 2019.

In this study, we compare the pathological mitosis rates, chromosomal abnormalities and nucleolar organizer activity in *Pinus pallasiana* D. Don seedlings from natural population in the Crimean Mountains and from the urban plantations in the steppe of Ukraine. On the stages of anaphase and telophase of mitosis, such chromosomal abnormalities as bridges were most often found in the seeds of plantations exposed to air pollutants, whereas lead and agglutination of chromosomes were found in seeds from iron ore dump stands. Our studies have shown that *P. pallasiana* can be used for genotoxic monitoring of technogenic polluted lands.

Key words: *Pinus pallasiana*, cytogenetic abnormalities, nucleolar activity, technogenic lands, air and soil pollution.

Introduction

Analysis of the range and frequency of cytogenetic abnormalities in the mitotic division of living organism cells is used to assess the genotoxic effects of the technogenic environments (Akinboro et al., 2011; Wang et al., 2014; Pekol et al., 2016; Koca et al., 2016). Such investigations on natural objects are relevant in industrial regions and centres in the steppe zone of Ukraine, occupying 40% of its total area. Actually, more than 169 million m^3 of industrial waste is annually produced in the course of production process in Krivoy Rog region. This small region is specializes in iron ore production. The area of quarries, mines, concentrating mills and sludge dumps located there is 35 thousand ha, including over 7 thousand ha of iron ore dumps. In addition to iron, these dumps contain precious and rare earth metals. Environmental man-induced migration of heavy metals in Krivoy Rog causes the so-called 'metal press' on the human body and other elements of the biota, acting as a destabilization factor of their living environments (Lysyi et al., 2007).

Coke and steel plants, coal-fired thermal power plants, mining dumps produce 91% of total emissions in the industrialized Donetsk region. The largest percentage of industrial emissions falls to the share of Mariupol (20.5%) and Donetsk (9.1%). Carbon monoxide (31.3%), methane (23.2%), sulphur dioxide (24.3%), nitrogen oxides (6.6%) dominate among the air pollutants. In addition, the atmosphere of Mariupol and Donetsk is contaminated by dust, phenol and formaldehyde. There are areas with high soil contamination with mercury (2.6 to 2.8 mg/kg), lead (145.4 mg/kg), manganese (5438 mg/kg), and chromium (1012 mg/kg) in Mariupol. A considerable amount of dust emitted to the atmosphere falls to the share of Novoamvrosievka cement plant (42.8% of total dust emissions in the Donetsk region). Basic components of the cement dust are calcium oxides (up to 60%), silica dioxides (up to 20%) and oxides of metals (up to 20%) (Kumar et al., 2008; Mishra, Siddiqui, 2014; Genisel et al., 2015). There are cancerogenic and mutagenic substances in the emissions of industrial enterprises in Donbass and Krivbass, for example, benzapyrene, which is not controlled by any sanitary and epidemiological service. In addition, rare earth metals, extracted from the soil and then dumped as a waste, contribute to the background radiation (Nouri, Haddioui, 2016). Qualitative and quantitative heterogeneity of physical and chemical agents in polluted and degraded lands makes it possible to evaluate specific genotoxicity of these agents. Conifers are often subjected to such a research (Geras'kin et al., 2005; Kalashnik, 2008; Korshikov et al., 2012; Kalashnik, Yasovieva, 2012). *Pinus pallasiana* D. Don, rather widespread and successfully growing in industrial regions of Ukrainian steppe, including Krivoy Rog iron ore dumps, is applicable in cytological and physiological studies (Bessonova, Grytsay, 2018). Dominating air pollution in Donetsk region or soil pollution (iron ore dumps) in Krivoy Rog region is likely to ambivalently influence both the frequency of cytogenetic aberrations and their range in living organisms.

The aim of our study is to analyse cytogenetic abnormalities in *P. pallasiana* under conditions of different types of industrial and edaphic environmental pollution to determine their specific effects.

Material and methods

P. pallasiana seedlings of seeds sampled from four urban plantings in the Ukrainian steppe were the objects of our research. The first plantation (Mariupol) is exposed predominantly to the emissions of metallurgical industry; the second one (Novoamvrosievka) is within a kilometre from the Europe's biggest cement plant, the third plantation (Donetsk) is located along the heavily trafficked road; the fourth one (Krivoy Rog) is found on the Pervomaysky iron ore dump of the Severny (Northern) Mining and Concentrating Mill (background air pollution). Age of plants in these plantations ranged from 25 to 40 years. Seeds were collected separately from 25 to 30 trees in plantations. As control, we used seeds from natural population in the Crimean Mountains (near the settlement of Nikita), collected from 40 trees aged 80 to 100. We studied seed progenies for cytogenetic effects, such as the range and rate of pathological mitosis, chromosomal abnormalities, and nucleolar activity.

Analyses were performed on temporary preparations of meristematic tissues of seedling roots (seeds sampled from each tree). The seeds were germinated in Petri dishes on wet filter paper in an oven at the temperature of 23 to 25 °C. Roots were fixed in acetic ethanol (1:3) and then stained with aceto-orcein. We used 50% solution of silver nitrate for staining of the nucleoli (Hozak et al., 1992; Treré, 1994). After staining, we rinsed the roots and prepared squashed preparations by the standard method (Pausheva, 1980). Micropreparation analysis was performed using a *Carl Zeiss Primo star* microscope (40x10 zoom) and *Axio Vision* Rel. 4.7 software. To carry out the analysis of mitosis, we counted all the cells at the stages of anaphase and telophase registering the percentage of cells with abnormalities. We classified mitotic pathologies and chromosomal abnormalities according to Bochkov et al. (1972) and

Glinska (2007), (Bochkov et al., 1972; Glinska et al., 2007). The number of nucleoli was counted in 1000 cells from each sample. The functional state of nucleolar organizer was evaluated according to nucleoli number, their sizes and the value of nucleus-nucleolus ratio. Conditional areas of nuclei and nucleoli were determined by their projections and the ratios of these areas were calculated.

Results and discussion

Lagging and lead of some chromosomes and less frequently multipolar mitosis were the major types of pathological mitosis found in seedling roots of *P. pallasiana* seeds at the stages of anaphase and telophase. Only lagging and lead were detected in plant seeds from control Crimean population and Donetsk plantation whereas a full range of mitotic abnormalities was observed in seeds from Mariupol and Krivoy Rog plantations (Table 1). Multipolarity is considered to be a specific response to heavy metal effects (Geras'kin et al., 2005; Belousov et al., 2012), present in excess among pollutants of these cities' environments. It should be noted that the progeny of *P. pallasiana* growing in an iron ore dump has the highest frequency of occurrence of all the three mitosis pathologies, namely lead (2.23%), lagging (0.49%), multipolarity (0.02%). The frequency of mitosis abnormalities in seed root cells was by times higher in technogenic ecotopes: by 30.4 times (in Krivoy Rog), by 4.4 times (Donetsk), by 3.1 (Mariupol) and by 2.1 times (Novoamvrosievska) than in the progeny of a Crimean natural population. Evidently, this fact is indicative of the specific effects of different qualitative types of pollution.

We have revealed four types of chromosomal abnormalities: bridges, chromosome fragmentation, ring chromosome and agglutination in seed root cells of the study plant samples. Only two types of abnormalities were found in the progeny of Crimean population, whereas all the four were present in the plants of each plantation (Table 1).

Such abnormalities as bridges were most often represented in the progenies of population and three plantations exposed mostly to air pollutants (0.03% in a population, 0.61% in Novoamvrosievka, 0.92% in Donetsk and 1.25% in Mariupol plantations). Along with a high percentage of bridges (1.27%), such a type of chromosomal abnormalities as agglutination (1.52%) was also predominant in the seed progeny of *P. pallasiana* plants growing in an iron ore dump. An increased frequency of such a rare abnormality as ring chromosome (0.1%) was also a specific feature of this plantation's progeny. This chromosomal abnormality was either absent or by order lower in other stands. Chromosomal abnormalities were more common than pathological mitosis in plant progenies from three plantations, exposed to the man-induced emissions. For example, the chromosomal abnormalities were, by many times, more frequent than the pathological mitosis in Novoamvrosievska, Donetsk and Mariupol plantations (by 3.4, 2.4 and 4.9 times, respectively). Just the converse effect was observed in the plant progeny from natural population, where pathological mitosis was 2.3 times more commonly present. We observed no significant differences in the frequencies of pathological mitosis and chromosomal abnormalities in the plant progenies from iron ore dump plantation. In general, these cytogenetic pathologies were much more common in seed progenies of *P. pallasiana* plantations from technogenic polluted lands (by 6.5 times in Novoamvrosievska, by 10.4 times in Donetsk, by 12.6 times in Mariupol and by 43.4 times in Krivoy Rog plantations), than in the plant progenies of natural population. It is obvious that cytogenetic abnormalities occurred by 3.4–6.7 times more often in plants from an iron-ore dump, than

T a b l e 1. Cytogenetic abnormalities in seedling root cells of *Pinus pallasiana* D. Don from the Crimean natural population and urban plantations in the steppe of Ukraine.

Cytogenetic indices	The population near the settlement of Nikita	Urban plantations			
		Novoamvrosievka	Donetsk	Mariupol	Krivoy Rog
The number of the investigated dividing cells, units					
10083	7175	6748	6774	12659	
The number of cells with pathological mitosis, units					
Lagging	2	2	14	12	62
Lead	7	12	13	5	282
Multipolar mitosis	0	0	0	2	3
Pathologies rates, % (M ± m)	0.09 ± 0.03	0.19 ± 0.05	0.40 ± 0.07**	0.28 ± 0.06**	2.74 ± 0.14**
The number of cells with chromosome abnormalities, units					
Bridges	3	44	62	85	161
Fragment	0	0	1	1	0
Ring chromosome	1	1	1	0	13
Agglutination	0	1	0	6	193
Chromosomal abnormalities rates, % (M ± m)	0.04 ± 0.02	0.64 ± 0.09***	0.95 ± 0.13***	1.36 ± 0.14***	2.89 ± 0.15***
The rate of all abnormalities, % (M ± m)	0.13 ± 0.03	0.84 ± 0.10***	1.35 ± 0.15***	1.64 ± 0.14***	5.64 ± 0.20***

Notes: from now on the variations from control are significant at ** – P ≤ 0.95; *** – P ≤ 0.999.

in three other stands of industrial cities. This fact can be accounted for by the high pollution of the overburden dump rock by heavy metals, including rare earth ones, contributing to the high background radiation and accumulated by plants. Laboratory studies on the root apical meristem cells of onion (*Allium cepa* L.) seedlings have shown that the salts of cadmium, lead, nickel, aluminium, copper and zinc (concentrations of 10^{-6} to 10^{-3} M) caused various cytogenetic damage: fragmentation, stickiness and lagging of chromosomes, chromosome bridges, multipolar anaphases, K-mitoses and chromosome condensation abnormality (Kalaev, Karpova, 2003; Stevens et al., 2007; Wang et al., 2014). The increased rates of these disorders were detected in plant seedlings of four coniferous species from technogenic polluted areas of the Southern Urals. Scots pine (*Pinus sylvestris* L.) was the most highly sensitive among these species, the frequency of mitotic abnormalities in its seeds ranging from 1.40 to 19.88% in different ecotopes. In seed progenies of three other species, this range was as follows: 2.6 to 6.8% in *Picea obovata* Ledeb., 4.34 to 6.8% in *Abies sibirica*, 4.0 to

8.6% in *Larix sucaszewii* Dyl. (Kalashnik, 2008). The number of chromosomal anomalies detected at the stage of metaphase was 7.5 to 16.05 times higher in seedling roots of these four conifer species' seeds from the polluted lands of the Southern Urals, than in the progenies of control stands. In addition, four to five types of anomalies are found in the seeds of conifers under polluted conditions, whereas no more than one to three types are typically found in plants under background conditions (Dovgalyuk et al., 2001; Kalashnik, 2008; Belousov et al., 2017).

An increased frequency of pathological mitoses (5.7%) in the seed progeny of *Betula pendula* in the Voronezh region is associated with air pollution with benzapyrene. Basing on comparisons of *Pinus sylvestris* seed progenies in the Voronezh regional nature reserves and technogenic polluted areas inclusive of the site within one kilometre from Novovoronezhskaya Nuclear Power Plant, the authors consider mitosis pathologies below 5% in the seedling roots to be the norm for assessing seed quality and using this species as a test object for cytogenetic monitoring of the environments (Butorina et al., 2002).

Cytogenetic abnormalities in the higher plants' cells induced by mutagens depend not only on their chemical composition (Liu et al., 1993; Butorina et al., 2001; Shkarupa et al., 2010), but also on their optical stereoisomerism. For example, S (+) stereoisomers of nitrosoalkylurea show twice higher activity in relation to seedling chromosomes of winter soft wheat (*Triticum aestivum* L.), than (R-) stereoisomers. The frequency of typical chromosome aberrations in seedling anaphase cells of *T. aestivum* depends on mutagen, its applied concentration, the variety genotype and makes 5.1 to 30.8% (Morgun et al., 2011).

The nucleolar activity is an important indicator of cell metabolic activity level (Haidarova, Kalashnik, 1999; Smolinski et al., 2007; Boulon et al., 2010; Stepinski, 2014). We revealed some differences in the activity of chromosome nucleolar organizer in seedling cells of the seeds from different *Pinus pallasiana* tree stands (Crimea, Donetsk and Krivoy Rog regions). For example, cells with up to 11 nucleoli were found in the seedlings of the natural population (Table 2).

We have found cells with the same number of nucleoli in the seed progeny of four tree plantations or with even higher number in some of them (Mariupol and Krivoy Rog plantations). There is a tendency towards decrease in the number of two-, three- and four-nucleolar cells in seedlings of the seeds from plantations (with the only exception for the one in Novoamvrosievka) in comparison to the control population. The percentage of cells with 3 to 7 nucleoli was 92.1% in the plant progenies of population, whereas the majority of seedling cells (80.1 to 85.4%) of the seeds from plantations had from 4 to 7 nucleoli. There were, on an average, 5.2 nucleoli in the seedling cells of the plant seeds from population and there were more (by 6.9 to 12.7%) nucleoli in seed progenies from plantations.

The largest nuclei, nucleoli and the lowest nucleus-nucleolus ratios are characteristic of seedling cells of plant seeds from *P. pallasiana* population in Crimea (Table 3). The mean nuclear area of seed progeny from Crimean population was significantly (by 26.2%) smaller only than that of the progenies of the Donetsk plantation. The nucleolar area of seed progeny from the above mentioned population was smaller only than that of the seedlings of the seeds from the same plantation (by 33.9%) and of the seeds from the Krivoy Rog plantation (by 48.6%). Nucleus-nucleolus ratios were higher in the seedlings of the seeds from planta-

T a b l e 2. The frequency of interphase nuclei with different number of nucleoli in seedling root cells of *Pinus pallasiana* D. Don from Crimean natural population and urban plantations in the steppe of Ukraine.

Sample	Frequency of nuclei with different nucleoli number, %												Mean number of nucleoli per nucleus
	The number of nucleoli per nucleus												
	1	2	3	4	5	6	7	8	9	10	11	12	
Crimean Mountains													
The population near the settlement of Nikita	0.3	2.3	10.7	18.9	23.6	28.1	10.8	3.3	1.6	0.3	0.1	0	5.20 ± 0.05
Donetsk region													
Novoamvrosievka plantation	0	0	5.6	19.8	23.8	27.5	14.3	6.3	2.0	0.5	0.2	0	5.56 ± 0.05***
Mariupol plantation	0	1.8	6.1	11.2	24.1	29.6	15.2	6.7	4.4	0.5	0.3	0.1	5.74 ± 0.05***
Donetsk plantation	0.3	1.77	4.5	13.9	22.1	26.0	18.2	8.8	2.9	1.3	0.16	0	5.86 ± 0.05***
Dnepropetrovsk region													
Krivoy Rog plantation	0	1.8	6.6	13.8	23.1	24.9	18.7	6.6	3.3	1.0	0.1	0.1	5.69 ± 0.05***

T a b l e 3. Nucleus-nucleolus ratio in *Pinus pallasiana* D. Don seedlings from Crimean population and urban plantations in the steppe of Ukraine.

Sample	Mean area of a nucleus mkm^2		Mean area of the nucleoli in a nucleus, mkm^2		Nucleus-nucleolus ratio	
	M ± m	CV, %	M ± m	CV,%	M±m	CV,%
The population near the settlement of Nikita	192.6 ± 10.2	37.5	28.62 ± 1.4	33.7	7.24 ± 0.4	42.1
Novoamvrosievka plantation	167.2 ± 5.8*	45.0	26.05 ± 1.7	24.1	8.21 ± 0.8	65.1
Mariupol plantation	188.6 ± 8.8	33.0	24.65 ± 1.4*	39.7	8.59 ± 0.6	42.2
Donetsk plantation	142.2 ± 7.57***	37.7	18.91 ± 0.8***	35.7	7.73 ± 0.3	53.5
Krivoy Rog plantation	192.1 ± 7.8	35.3	14.70 ± 1.1***	36.8	15.54 ± 1.0***	50.1

tions in comparison with the plant progenies of the population by 6.8% (Donetsk), 13.4% (Novoamvrosievska), 18.6% (Mariupol) and 11.46% (Krivoy Rog). The increase in this ratio in seed progenies of plants exposed to technogenic emissions is associated with the smaller nuclear and nucleolar areas in seedling roots (Treré, 2000). A marked increase of the nucleus-nucleolus ratio in seed progeny of plants growing in the iron ore dump is primarily predetermined by significantly smaller sizes of the nucleoli, than in control. Consequently, air and edaphic technogenic pollution increases the activity of chromosome nucleolar organizers in seed progenies of *P. pallasiana*.

In the course of laboratory studies of radon effects on *Zebrina pendula* Schirt., the nucleolar activity has turned out to be the most sensitive cytogenetic indicator (Butorina, Kalaev, 2000; Boulon et al., 2010). A high sensitivity of seed progeny of some conifer species to industrial pollution in the Southern Urals manifested itself through the higher rates of chromosomal disorders at the stages of anaphase and telophase of mitosis (Kalashnik, 2008). In the course of our studies, we have found chromosomal abnormalities to be the most sensitive cytogenetic indicator in the progeny of three *Pinus pallasiana* plantations, exposed to air pollutants, bridges clearly dominating among these abnormalities. Pathological mitosis and chromosomal abnormalities occurred with the same frequency in the progenies of plants growing in the ore-mining dump, lead and agglutination of chromosomes being predominant. This fact is indicative of the specific effects of edaphic and air pollution.

Though the frequency of chromosome aberrations in the cells of living organisms is widely used to evaluate the genotoxic effects of various physical and chemical agents on living organisms, the mechanisms of these mitosis anomalies' occurrence are poorly investigated so far (Harvey et al., 1997). All types of chromosomal aberrations are considered to be one phenomenon, as their common bases are the DNA one- and double strand breaks (Bryant, 1997). However, various types of chromosomal aberrations are caused by a different number of molecular genetic events involving various morphological regions of chromosomes, which significantly differ in their structural and functional characteristics. In addition, certain individual chromosomes within karyotype may differ significantly from each other in their participation rates in chromosomal aberrations (Richardson et al., 1998). Chromosomal aberrations are caused by some non-mutagenic chemicals, as well as metabolic poisons, which inhibit DNA synthesis and induce DNA double breaks. The occurrence of chromosomal aberrations depend on the specific genotype characteristics of an individual, controlling the functional activity of the proteins, which provide packing of primary DNA sequences (Morgan et al., 1998). Disintegration of the cell plasmatic membranes results in chromosomal aberrations. A low-intensity prolonged exposure to radiation causes a decrease in the membrane phospholipids' content; thus, changing its physical and chemical properties (viscosity and permeability), which in its turn violates the normal functioning of the cell, including its repair processes (Wojcik et al., 1996).

Cytogenetic abnormalities in seed progenies of woody plants in industrial regions result either from direct adverse effects of toxic air and soil pollutants, or from the accumulation of these pollutants, mutagens and heavy metals within the plant organs and their transfer to seed buds. On the other hand, higher frequencies of cytogenetic abnormalities in seed progenies may be caused by pathologic meiosis and seed bud fertilization with anomalous pollen. Pathologic meiosis associated with cytogenetic anomalies is observed in natural populations (Jeelani et al., 2013).

Thus, we have detected high frequency of cytogenetic abnormalities (pathologic mitosis, chromosomal aberrations and nucleolar organizer activity changes) in *P. pallasiana* seedlings of seeds from industrial regions of Ukrainian steppe (Donetsk and Krivoy Rog region). The highest rates of these abnormalities were characteristic of plant seed progenies from Krivoy Rog ore mining dumps and sites near Donetsk metallurgical enterprises where soil substrates and air is contaminated by heavy metals. Specific cytogenetic abnormalities in seed progenies are associated with dominating pollution type.

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sciendo

Ekológia (Bratislava) Vol. 38, No. 2, p. 126–139, 2019
DOI:10.2478/eko-2019-0010

DYNAMICS OF SELECTED SOIL QUALITY INDICATORS IN RESPONSE TO LAND USE/COVER AND ELEVATION VARIATIONS IN WANKA WATERSHED, NORTHWESTERN ETHIOPIAN HIGHLANDS

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Abstract

Abera W., Assen M.: Dynamics of selected soil quality indicators in response to land use/cover and elevation variations in Wanka watershed, northwestern Ethiopian highlands. Ekológia (Bratislava), Vol. 38, No. 2, p. 126–139, 2019.

Soil quality (SQ) dynamics assessment vis-à-vis land use/land cover (LULC) and elevation variations in Ethiopia is desirable as elevation impact on land use is highly pronounced. This study examined SQ indicators dynamics across LULC and elevation variations. For this, surface soil samples (0–20 cm) were collected from the recognized LULC categories of different elevations in Wanka watershed, northwestern Ethiopian highlands. Both disturbed and undisturbed soil samples that were taken from three adjacent LULC (natural forest, grazing and cultivated lands) and elevation (2238–2300, 2400–2600, and 2700–2800) classes analysed for the selected physico-chemical SQ indicators. Two-way ANOVA, Tukey's multiple comparison test and SQ deterioration index were computed. The impact of LULC and elevation was found significant on key SQ indicators. In cultivated and grazing lands, soil organic matter (SOM) and soil nutrients like total nitrogen declined significantly ($p < 0.01$). Conversely, bulk density increased significantly ($p < 0.01$). The divalent basic cations (Ca^{2+} and Mg^{2+}), cation exchange capacity and pH significantly ($p < 0.01$) decreased in upper elevation. Synergetic effect of LULC and elevation variations was found significant ($p < 0.01$) on SOM, total nitrogen, available phosphorus, water content at field capacity and soil particle distribution (silt and clay). Thus, elevation specific land management strategies that improve these SQ indicators need to be emphasized.

Key words: soil quality, soil quality deterioration index, cultivated land, grazing land, natural forest land.

Introduction

Soil quality (SQ) assessment in light of land use variation is very critical in tropical regions to improve land-use decisions (Sağlam et al., 2015; Yimer et al., 2006; Fu et al., 2000). In this region, conversion of natural land covers especially natural forests into cultivated land (CL) is very intense and eventually result in SQ deterioration (Guillaume et al., 2016; Islam, Weil, 2000). Land use/land cover (LULC) change in sub Saharan African region has brought failure of soils to provide essential ecosystem services and subsequently SQ decline has been a major challenge (Diao et al., 2010). In this context, SQ can be considered as the capacity of soil to function in response to land management and stress made by natural and human induced factors, which can be evaluated using SQ indicators (Schjønning et al., 2004). It consists of physical, chemical and biological indicators that react to changes in soil conditions (Sağlam et al., 2015).

The issue of SQ in Ethiopia is very critical as soil resource has been under great stress due to human population and livestock pressure, which eventually results in over exploitation and mismanagement for several decades (Gelaw et al., 2015; Eyayu et al., 2009). A significant amount of CL is under severe nutrient depletion and physical degradation due to unsustainable land use system like expansions of CL to fragile lands (Teferi et al., 2016; Gelaw et al., 2015; Teshome, 2013).

LULC variation is the foremost factor of SQ dynamics at catchment scale (Wang et al., 2001) and information on responses of SQ indicators is essential to develop sustainable land management plan (Ayalew, Kassahun, 2016; Karlen et al., 2003). However, this issue has been given little attention as great emphasis has been given to physical soil erosion problem for several decades (Karlen et al., 2003).

The impact of LULC and elevation variations on SQ indicators is site specific (Teferi et al., 2016), and hence, there is discrepancy in the findings. Some studies revealed that LULC variation has adverse impacts on SQ indicators (Gebrelibanos, Assen, 2013; Asmamaw, Mohammed, 2013; Lemenih, Itanna, 2004). Conversely, some others reported that LULC variation has no impact on some SQ indicators. For example, Ashagrie et al. (2005) reported that soil organic carbon and total nitrogen have shown no change after the conversion of natural forest to eucalyptus plantation in Munesa area of Ethiopia. Similarly, despite authors like Ayalew and Kassahun (2016) and Hussien et al. (2015) reported that clay content was significantly higher in CL, others (Eyayu et al., 2009; Woldeamlak, 2003) reported lower clay content in CL. Likewise, while Guteta and Abegaz (2017) and Teshome (2013) reported higher available phosphorus in forest lands, others like Asmamaw and Mohammed (2013) reported that it is higher in CL. Moreover, several studies that have been conducted on SQ dynamics in Ethiopia overlooked soil-LULC and elevation interaction (Abegaz et al., 2016; Teferi et al., 2016; Asmamaw, Mohammed, 2013; Gebrelibanos, Assen, 2013; Teshome, 2013; Tesfahunegn, 2013; Eyayu et al., 2009; Ashagrie et al., 2005; Lemenih et al., 2005; Lemenih, Itanna, 2004). These studies emphasized on LULC dynamics and slope aspect impact on SQ indicators. Therefore, the present study was aimed to assess the effect of LULC and elevation variations, and their interactions on selected SQ indicators in Wanka watershed, northwestern Ethiopian highlands.

Materials and methods

Description of the study area

Wanka watershed lies between 11^{0}29’24” and 11^{0}42’36” North latitudes, and 37^{0}58’12” and 38^{0}14’24” East longitudes (Fig. 1). It is one of the head streams of the Blue Nile (Abay) basin and covers a total area of 252 km^2. The watershed

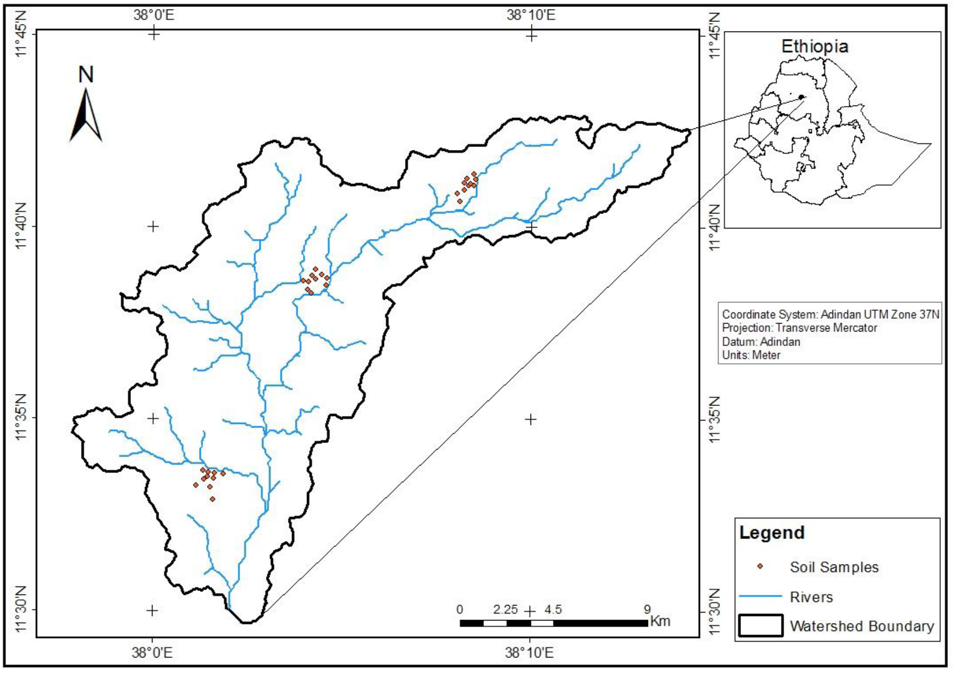


Fig. 1. Location of the Wanka watershed.

is a part of the extensive Afro-Arabian plateau, which is characterized by the uplifting of landmasses and out pouring of lava (Mohr, 1971). Like that of the other headstreams of Blue Nile (Abay) basin, it is characterized by diverse topographic conditions. The elevation ranges from an altitude of 2,238 to 4,086 meter above sea level (m.a.s.l) and it experiences subtropical to Alpine climatic conditions. The soil units of the watershed are Chromic Luvisols (41.1%), Eutric Leptosols (34.12%) and Haplic Luvisols (24.84%) (FAO, 1990). The natural vegetation of the watershed include grass, bushes, natural and plantation trees (*Eucaluptus globulus* and *Cupressus lusitanica*). The main natural tree species include: weyra (*Olea africana*), yabesha tid (*Juniperus procera*) and yabesha girar (*Acacia abyssinica*).

The mean annual temperature of Wanka watershed is 17.3 °C. The mean annual minimum and maximum monthly temperature are 8.4 and 26 °C, respectively. The annual rainfall recorded for the years 1994 through 2015 revealed that the mean annual rainfall of the watershed is 1320 mm. The rainfall pattern is unimodal with one major (summer) rainy season, which extends from June to August, and sometimes it extends up to September. About 80% of the total annual rainfall takes place from June to August, peaking in July (369.4mm) (NMSAE, 2015).

Soil sampling

Three adjacent LULC types (natural forest, cultivated and grazing lands) that were in similar slope gradient both in the upper, middle and lower part of the watershed were selected for soil sampling (Table 1). The elevation of the sampled sites ranges were 2238–2300 m a.s.l (lower), 2400–2600 m a.s.l. (middle) and 2700–2800 m a.s.l. (upper). According to Hurni (1998), the agro ecological zones of Ethiopia include: weyna dega (mid altitude, 1300–2600 m a.s.l.), dega (high-altitude, 2600–3400 m a.s.l.), high dega (high-altitude, 3400–3800 m a.s.l.), and wurch (> 3800 m a.s.l.). Cultivated and grazing lands had been part of the adjacent forest cover before several years (information from key informants). The bench mark natural forest in both elevation classes mainly consisted of *Juniperus procera* (yabesha tid) and *Olea europaea* (Woyera). Moreover, the sampled CL in the 3 elevation classes of the watershed were under the cultivation of Teff (*Eragrostis abyssinica*).

T a b l e 1. Description of major LULC types in the Wanka watershed, north western highlands of Ethiopia.

Land use/cover type	Description
Cultivated land	Land that has been used for annual crops cultivation.
Natural forest land	Land use type in which human interference is very minimal and covered with thick growth of natural vegetation (trees and undergrowth).
Grazing land	Land that serves as common grazing land. It is void of big vegetation like tress, bushes rather it predominantly covered by very short grass.

A composite soil sample with 5 subsamples in 10 m * 10 m plot size was collected at each site. Four replications of cultivated land, as it has the largest area coverage, and three replications each for natural forest and grazing lands were sampled at each elevation class. A total of 30 disturbed samples were collected after the harvesting period (January) at surface depth (0 to 20 cm) using auger. Topsoil is the most common sampling depth for soil testing as it contains significant proportion of soil nutrients and is very sensitive to land use changes (Abegaz et al., 2016; Andrews et al., 2002). Sub samples were mixed thoroughly (clods carefully crushed) and approximately half to one kg of composite sample from each sample plot was prepared, put in plastic bag, labelled and carried to the laboratory for analysis. In addition, the undisturbed core samples from each sampled land use type in the respective elevation classes were taken at the centre of each plot with cylindrical metal samplers (5 cm length with 5 cm diameter) for bulk density (BD) analysis.

Soil analysis

Sixteen SQ indicators were analysed at Adet Agricultural research centre soil laboratory, Ethiopia following standard soil laboratory analysis procedures. Soil water content at field capacity (FC) and permanent wilting point (PWP) were determined at 1/3 and 15 bars, respectively, by pressure membrane suction method and available water content (AWC) was determined by calculating their differences (Estefan et al., 2013). Bouyoucos hydrometric and core sample method were respectively used to determine the soil particle size distribution and BD (Estefan et al., 2013). Total porosity was calculated by assuming a particle density of 2.65 g cm^{-3}, that is, P = (1-BD/PD) * 100, where P = total porosity (%), BD = the bulk density (g cm^{-3}) and PD = particle density *(gcm^{-3})* (Landon, 1991). Soil pH was measured in 1:2.5 soil–water ratio suspension (Van-Reeuwijk, 2002). As suggested by Carter and Gregorich (2006), Kjeldahl, Olsen and Walkley-Black oxidation methods were respectively used to determine soil total nitrogen (TN), available phosphorus and organic carbon. Soil organic matter (SOM) was determined by multiplying organic carbon (%) by a constant 1.724 (Carter, Gregorich, 2006). Cation Exchange Capacity (CEC) and exchangeable bases (K^+, Mg^{2+}, Ca^{2+} and Na^+) were examined using ammonium acetate extraction method (at pH 7) as described in Estefan et al. (2013). Exchangeable K^+ and Na^+ were measured by flame photometer, and Ca^{2+} and Mg^{2+} using atomic absorption spectrometry. Then, the percentage of base saturation was calculated by dividing the summation of exchangeable cations to CEC and multiplied by 100 (Estefan et al., 2013).

Statistical analysis

Two-way ANOVA was computed to test the effect of variations of LULC and elevation, and their interactive effect on mean values of the selected SQ indicators. Tukey's post hoc multiple comparison test was used to differentiate mean variation of SQ indicators that showed statistically significant differences in the analysis of variance. Statistical analysis was performed using SPSS version 23.

Soil quality deterioration index

SQ deterioration index (DI) of each selected SQ indicator obtained by computing the percentage of difference between mean value of each selected SQ indicators under different LULC and adjacent bench mark soil (Gui et al., 2009; Lemenih et al., 2005; Islam, Weil, 2000). Then DI of each selected SQ indicator were added and averaged to obtain the cumulative DI (Lemenih et al., 2005; Wang et al., 2001). Out of the sixteen measured SQ indicators, porosity, BD, SOM and soil nutrients (available phosphorus, TN, CEC, Ca^{2+}, Mg^{2+} and K^+) were selected to calculate DI based on experts' opinion (Andrews et al., 2002), and these SQ indicators are very sensitive to land use changes and often used to compute DI (Gui et al., 2009).

Results

Physical SQ indicators

Particle size distribution

The overall distribution of soil separates in the study watershed significantly ($p < 0.01$) varies across LULC and elevation variations, but their interactive effect was significant ($p < 0.01$) merely on silt and clay contents (Table 3). While sand and clay contents were significantly higher in CL than NFL ($p < 0.01$) and GL ($p < 0.05$), silt content was found significantly ($p < 0.01$) higher in NFL than other LULC types, and in GL than CL ($p < 0.05$) (Tables 2 and 4).

T a b l e 2. Mean values of the selected SQ indicators across LULC and elevation classes of the Wanka watershed, north western highlands of Ethiopia.

SQ indicators	LULC classes			Elevation classes		
	CL	NFL	GL	Upper	Middle	Lower
Sand (%)	35	28	33	35	33	29
Silt (%)	29	42	34	34	35	36
Clay (%)	36	30	33	31	33	35
BD (g/cm^3)	1.28	0.98	1.18	1.1	1.19	1.22
Total Porosity (%)	52	63	56	60	55	54
FC (%)	44	51	42	44	48	45
PWP (%)	22	24	26	22	25	25
AWC (%)	22	27	16	23	23	20
pH H_2O (1:2.5)	5.9	6.5	6	5.9	6.2	6.3
SOM (%)	2.3	7.2	3.9	4.8	3.5	4.4
Av. Ph (ppm)	11.6	12.3	5.3	4	21	4.7
TN	0.20	0.59	0.25	0.42	0.27	0.31
C/N ratio	11.7	12.7	15.4	12.8	12.9	13.5
CEC ($Cmol_c\ kg^{-1}$soil)	33.8	43.1	34.6	30.6	37.6	42.4
Ca^{2+} ($Cmol_c\ kg^{-1}$soil)	23.9	32	22.5	20.9	26.3	30.8
Mg^{2+} ($Cmol_c\ kg^{-1}$soil)	5.3	6.4	5.6	4.5	5.5	7.1
K^+ ($Cmol_c\ kg^{-1}$soil)	0.82	0.82	0.81	0.82	0.82	0.80
Na^+ ($Cmol_c\ kg^{-1}$soil)	0.13	0.12	0.14	0.13	0.13	0.13
PBS (%)	91	92	85	86	89	91

Notes: SQ – soil quality; CL – cultivated land; GL – grazing land; NFL – natural forest land; LULC – land use and land cover; BD – bulk density; Av.ph – available phosphorus; TN –total nitrogen; C/N – carbon nitrogen ratio; CEC – cation exchange capacity; Ca^{2+} – exchangeable calcium; Mg^{2+} – exchangeable magnesium; K^+ – exchangeable potassium; Na^+ – exchangeable sodium; PBS – percent base saturation.

T a b l e 3. Two-way analysis of variance (ANOVA) result of the selected SQ indicators (0–20 cm depth) under three LULC and elevation classes of the Wanka Watershed, northwestern highlands of Ethiopia

SQ indicators	**LULC (df = 2)**		**Elevation (df = 2)**		**LULC * Elevation (df = 4)**	
	F-Statistic	**P-value**	**F-Statistic**	**P-value**	**F-Statistic**	**P-value**
Sand (%)	10.082	0.001**	6.612	0.005**	2.233	0.092 ns
Silt (%)	28.692	0.000**	1.024	0.373 ns	6.046	0.001**
Clay (%)	20.060	0.000**	6.234	0.006 **	16.880	0.000**
BD (g/cm^3)	34.625	0.000**	9.400	0.001 **	2.555	0.062 ns
Porosity (%)	33.653	0.000**	9.856	0.001**	2.567	0.061 ns
FC (%)	12.563	0.000**	1.833	0.179 ns	19.344	0.000**
PWP	4.216	0.025*	1.497	0.242ns	8.642	0.000**
AWC	33.484	0.000**	2.553	0.096 ns	9.793	0.000**
pH H_2O (1:2.5)	20.462	0.000**	9.856	0.001**	3.816	0.014*
SOM (%)	49.042	0.000**	5.429	0.010**	7.942	0.000**
Av. Ph (ppm)	22.061	0.000**	111.39	0.000**	17.326	0.000**
TN (%)	30.569	0.000**	3.772	0.036*	4.570	0.006**
CEC ($Cmol_c kg^{-1}$soil)	6.066	0.007**	8.253	0.002**	1.403	0.260 ns
Ca^{2+} ($Cmol_c kg^{-1}$soil)	10.102	0.001**	9.477	0.001**	2.193	0.097 ns
Mg^{2+} ($Cmol_c kg^{-1}$soil)	1.556	0.229 ns	10.751	0.000**	.434	0.783 ns
PBS (%)	11.375	0.000**	5.936	0.008**	2.341	0.081ns

Notes: ** – significant at $P < 0.01$; * – significant at $P < 0.05$; ns – not significant; df – degree of freedom.

Bulk density (BD) and Total porosity

The highest (1.28 g/cm^3) and lowest (0.98 g/cm^3) mean values of BD across all LULC and elevation classes of Wanka watershed were recorded in CL and NFL respectively. It was found significantly ($p < 0.01$) higher in CL than NFL and GL. Similarly, it was significantly higher in lower elevation than upper ($p < 0.01$) and middle ($p < 0.05$) elevation classes (Table 4). In contrast with NFL, BD increased by 30.6 % and 20.41% in CL and GL respectively (Fig. 2).

Field capacity (FC), permanent wilting point (PWP) and available water holding capacity (AWC)

Water retention capacity of soil at FC and AWC was significantly ($p < 0.01$) influenced by LULC variation. The interactive effect of LULC and elevation variation on both FC, PWP and AWC was also statistically significant ($p < 0.01$), but the effect of elevation position was found to be statistically insignificant ($p > 0.05$) (Table 3). Tukey's multiple comparison test (Table 4) revealed that FC was significantly ($p < 0.01$) higher in NFL than CL and GL. Conversely, while PWP was significantly ($p < 0.05$) higher in GL than CL, AWC was significantly ($p < 0.01$) higher in NFL than GL.

T a b l e 4. Results of Tukey's post hoc multiple comparisons test of statistically significant SQ indicators in the Wanka watershed, northwestern highlands of Ethiopia.

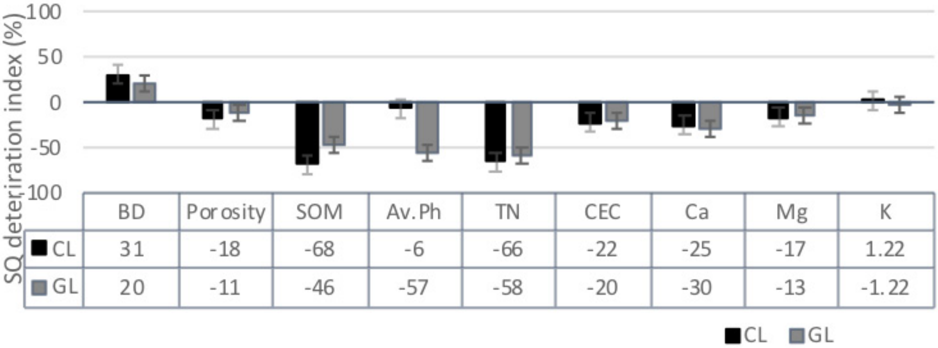
	LULC		Elevation position	
SQ indicators	Sig. contrasts of LULC classes	P-value	Sig. contrasts of elevation differences	P-value
Sand (%)	Cultivated and Natural forest lands	0.000**	Lower and upper	0.003**
	Grazing and Natural forest lands	0.017*		
Silt (%)	Cultivated and Natural forest lands	0.000**		
	Grazing and natural forest lands	0.000**		
	Cultivated and grazing lands	0.019*		
Clay	Cultivated and Natural forest lands	0.000**	Upper and lower	0.001**
	Cultivated and grazing lands	0.025*		
	Grazing and Natural forest lands	0.004**		
BD (g/cm^3)	Cultivated and Natural forest lands	0.000**	Lower and upper	0.001**
	Natural forest and Grazing lands	0.000*	Middle and upper	0.015*
Porosity (%)	Cultivated and Natural forest lands	0.000**	Lower and upper	0.001**
	Natural forest and Grazing lands	0.000*	Middle and upper	0.011*
FC	Natural forest and cultivated lands	0.002**		
	Natural forest and Grazing lands	0.000**		
PWP	Cultivated and grazing lands	0.020*		
AWC (%)	Cultivated and Natural forest lands	0.002**		
	Cultivated and Grazing lands	0.001**		
pH H_2O (1:2.5)	Cultivated and Natural forest lands	0.000**	Lower and upper	0.001**
	Natural forest and Grazing lands	0.000**	Middle and upper	0.008**
SOM (%)	Cultivated and Natural forest lands	0.000**	Upper and middle	0.008**
	Natural forest and Grazing lands	0.000**		
	Grazing and cultivated lands	0.012*		
Av. Ph (ppm)	Cultivated and Grazing lands	0.000**	Middle and lower	0.000**
	Natural forest and Grazing lands	0.000**	Middle and upper	0.000**
TN (%)	Cultivated and Natural forest lands	0.000**	Upper and middle	0.028*
	Natural forest and Grazing lands	0.000**		
CEC ($Cmol_c kg^{-1}$soil)	Natural forest and cultivated lands	0.015*	Lower and upper	0.001**
	Natural forest and Grazing lands	0.014*	Middle and upper	0.038*
Ca^{2+} ($Cmol_c kg^{-1}$soil)	Natural forest and cultivated lands	0.009**	Lower and upper	0.000**
	Natural forest and Grazing lands	0.001**	Middle and upper	0.041*
Mg^{2+} ($Cmol_c kg^{-1}$soil)			Lower and upper	0.000**
			Lower and middle	0.023*
PBS (%)	Cultivated and Grazing lands	0.001*	Upper and lower	0.003**
	Natural forest and Grazing lands	0.000*		

Notes: ** – significant at P < 0.01; * – significant at P < 0.05.

Chemical SQ indicators

Soil pH, Soil organic matter (SOM) and Total nitrogen (TN)

The pH value in Wanka watershed was found in the range of 5.9 and 6.5 (Table 2). It was significantly affected by LULC ($p < 0.01$), elevation ($p < 0.01$) and their interaction ($p < 0.05$) (Table 3). Similarly,



	BD	Porosity	SOM	Av.Ph	TN	CEC	Ca	Mg	K
CL	31	-18	-68	-6	-66	-22	-25	-17	1.22
GL	20	-11	-46	-57	-58	-20	-30	-13	-1.22

Fig. 2. Deterioration indices of the selected soil quality indicators for CL and GL in Wanka watershed, northwestern highlands of Ethiopia.
Notes: CL – cultivated land; GL – grazing land; SQ – soil quality.

LULC variation and their interactive effect on SOM was significant ($p < 0.01$). It was significantly ($p < 0.01$) higher in NFL than CL and GL, and in GL ($p < 0.05$) than CL (Tables 2 and 4). Compared with NFL, it decreased considerably (68%) in CL (Fig. 2). SOM also showed an increment in the upper elevation than middle and lower elevation of the study area. It was significantly higher ($p < 0.01$) in the upper elevation than the middle elevation. LULC ($p < 0.01$), elevation ($p < 0.05$), and their interaction ($p < 0.01$) also significantly affect TN (Table 3). TN in NFL (0.59%) was significantly ($p < 0.01$) higher than GL (0.25%) and CL (0.20%) (Tables 2 and 4). In CL and GL of Wanka watershed, the amount of TN rated as low and medium (Landon, 1991), respectively, and in reference to NFL, it declined by 66 and 58% in CL and GL, respectively (Fig. 2). It was also found significantly ($p < 0.05$) higher in the upper elevation than in the middle elevation (Table 4).

Available phosphorus (Av.ph) and Carbon nitrogen ratio (C/N)

Available phosphorus was significantly ($p < 0.01$) higher in NFL than GL, and in CL than GL (Table 4). In contrast to the soil under the NFL, it was reduced by 6 and 57% respectively in CL and GL. Available phosphorus was also significantly ($p < 0.01$) higher in the middle than other elevation classes. Conversely, the effect of LULC, elevation and their interactive effect was statistically insignificant on the C/N ratio. Except in the GL (15.4) of lower elevation, the C/N ratio in the Wanka watershed was found to be optimum (ranged 11.7 to 13.7) for most cultivated crops.

CEC, exchangeable cations and PBS

Cation exchange capacity (CEC) in NFL was found to be significantly higher than CL and GL at $p < 0.05$(Table 4). There was also significant increment of CEC in the lower elevation. Its mean value in upper, middle and lower elevation were 31 $Cmol_c\ kg^{-1}$soil, 39 $Cmol_c\ kg^{-1}$soil and 42 $Cmol_c\ kg^{-1}$soil, respectively (Table 2). It was significantly lower in the upper ($p < 0.01$) and middle ($p < 0.05$) elevation than in the lower elevation (Table 4).

Effect of LULC variation was found significant on Ca^{2+}, but not on Mg^{2+} and K^+. Exchangeable Ca^{2+} was significantly ($p < 0.01$) higher in soils under NFL than CL and GL (Table 4). In CL, while Ca^{2+} decreased by 25%, K^+ showed slight increment (1.2%). On the other hand, in the grazing land, K^+ and Ca^{2+} reduced respectively by 1.2 and 30% (Fig. 2). Similarly, despite it not being statistically significant, Mg^{2+} was found lower in CL.

Percent base saturation (PBS) in the study watershed was generally high (Landon, 1991). The mean value of PBS across all LULC and elevation classes ranged from 85 to 92% (Table 2). PBS was significantly ($p < 0.01$) higher in NFL and CL than in GL (Table 4). The factor may be the prevalence of relatively higher basic cations in these LULCs. On the other hand, PBS was significantly ($p < 0.01$) higher in the lower elevation than upper elevation.

Soil quality deterioration index

SQ deterioration indices (DI) result revealed that there was a declining trend of most of the selected SQ indicators in CL and GL. Highest negative DI values were mainly recorded for SOM (-68%) and TN (-66%) in CL, and available phosphorus (-57%) and TN (-58%) in GL. In contrast with other SQ indicators, the deterioration of available phosphorus in CL is lowest (Fig. 2). This is perhaps due to the application of Diammonium Phosphate (DAP) chemical fertilizer. DI of BD both in CL and GL disclosed SQ reduction (compaction increment). It increased by 31 and 20% in CL and GL respectively (Fig. 2).

Discussion

Soil particle size distribution

Silts are the most mobile elements by erosion, and hence, lower silt content in CL and GL implies erosion is high in these land uses, particularly in CL. On the other hand, sand and clay contents were found significantly ($p < 0.01$) higher in the upper and lower elevations, respectively (Table 4). This could be attributed to the clay removal by erosion, leaving behind sand fractions in the upper elevation and subsequently accumulated in the lower elevation. The impact of elevation variation on clay particles movement towards lower elevation might be enhanced by poor land management practices in the CL of upper elevation of Wanka watershed. Yimer et al. (2006) also reported a higher proportion of clay contents in lower elevation of CL in the south-eastern highlands of Ethiopia. Variation in the distribution of soil separates across LULC and elevation classes has its implication on the SQ. For example, high sand content in CL of Wanka watershed affects water holding capacity of a soil, and subsequently, water and nutrient availability for growing plants (Hussien et al., 2015).

Bulk density and total porosity

High BD in CL of lower elevation most likely arises from SOM deficiency as BD and SOM have an inverse relationship (Awotoye et al., 2011). In the study area, there was a significant

($p < 0.01$) inverse correlation between BD and SOM ($r = -0.72$) (data not shown). Higher BD in CL of Wanka watershed signifies the severity of adverse impact on rooting depth, soil porosity, infiltration, water and plant nutrient availability for crops. Porosity also varied significantly ($p < 0.01$) across both LULC and elevation variations, but their interactive effect was found insignificant ($p > 0.05$) (Table 3). Total porosity (63%) in NFL (Table 2) was found significantly ($p < 0.01$) higher than soil under CL and GL. Similarly, it was significantly higher in upper elevation than lower ($p < 0.01$) and middle ($p < 0.05$) elevation classes (Table 4). This explains that higher levels of SOM produce better proportions of total porosity. SOM maximize total porosity (Guo et al., 2016), and in Wanka watershed, significant ($p < 0.01$) positive association ($r = 0.71$) (data not shown) was observed between total porosity and SOM. Soil management induced problems like intensive tillage without appropriate soil management may accelerate SOM degradation and compaction.

Field capacity (FC), permanent wilting point (PWP) and available water holding capacity (AWC)

The possible factor for higher FC and AWC in NFL than CL and GL could be due to the prevalence of higher content of silt and SOM in NFL. Loam and silt loam soil (Asmamaw, Mohammed, 2013) and soil with high SOM content (Adugna, Abegaz, 2016) have high AWC. Moreover, despite it not being statistically significant, AWC is lower in CL than NFL. High AWC in the soil is very critical for plant growth and crop production, and hence, the measures that increase AWC (e.g., conservation) in LULC types other than NFL need to be practiced in Wanka watershed. Conservation regulates water infiltration and drain away from CL (Asmamaw, Mohammed, 2013).

Soil pH, Soil organic matter (SOM) and Total nitrogen (TN)

Significantly ($p < 0.01$) higher pH in NFL than CL and GL (Table 4) was observed in the study watershed. Relatively lower pH value in CL than other land uses in Wanka watershed may be resulted from the mining of basic cations by crops, intense ploughing and continuous application of chemical fertilizers. Continuous use of chemical fertilizer rich in nitrogen has an acidifying effect on soil (Savci, 2012). Despite the fact that the pH value in the CL was found lower than other land uses, it was found optimum for most crops. Hence, it has no adverse impact on SQ and agricultural productivity in the existing condition. According to Landon (1991), pH values between 5.5 and 7 is preferred for most crops. But the lower pH value in CL may potentially reduce SQ unless necessary measure is taken in the study area. Little removal of base forming minerals by erosion and recycling of nutrients by decomposition of basic cation rich plant residues bring higher pH value in NFL (Abrham et al., 2012). On the other hand, pH value was found significantly ($p < 0.01$) lower in the upper elevation than the lower and middle elevation classes. This might be due to the fact that soluble basic ions (Ca^{2+}, Mg^{2+}) migrate down to lower elevation due to erosion and leaching as rainfall increases with increasing altitude. In Wanka watershed, this might be accentuated by poor land management.

The amount of SOM in NFL, GL and CL of Wanka watershed respectively were rated as high, medium and low (Landon, 1991). Low SOM content in CL might be due to low input of organic fertilizers (crop residues and manure), rapid decomposition and mineralization of organic matter due to intensive tillage (Aghasi et al., 2011). This implies high soil nutrients depletion in CL as SOM is the foremost indicator of SQ. It determines structural stability, moisture retention, nutrient status and biological activity of the soil (Schjønning et al., 2004). Elevation difference also had a significant impact on SOM. It was significantly higher ($p < 0.01$) in the upper elevation than in the middle elevation, and this in turn resulted in higher TN in upper elevation. The most likely reason for high SOM could be low decomposition and mineralization rate of organic matter due to relatively low temperature condition. TN was found significantly lower in CL than the other LULC of the study area, perhaps due to crop uptake of nutrients and less return via organic fertilizers like crop residues and manure. On the other hand, relatively higher TN in GL than CL could be attributed to the recycling of nitrogen via animal waste of free grazing cattle (Ashagrie et al., 2005).

Available phosphorus (Av.ph) and Carbon nitrogen ratio (C/N)

According to Landon (1991), for most cereal crops and grass, the adequate amount of available phosphorus is > 8 ppm, but in the lower and upper elevation GL of Wanka watershed, it was found to be below the critical value. This may be due to overgrazing, which brings loss of phosphorus (due to compaction and erosion) and absence of any phosphorus inputs (fertilizers) that substitute this loses (Adugna, Abegaz, 2016). Conversely, significantly ($p < 0.01$) high available phosphorus in middle than upper and lower elevation classes could be attributed to the nature of parent material and the land management practice of the area. Presence of higher available phosphorus in NFL and CL than GL may also attribute to low mining of available phosphorus by existing plants (Woldeamlak, 2003) or due to the addition of phosphorus through the application of fertilizer in CL. Amount of available phosphorus in top soil layer is a function of intensity of land use, management practice, organic matter or microbial biomass and history of land use (e.g., application of artificial fertilizer) (Schjønning et al., 2004). C/N ratio in Wanka watershed was slightly higher in GL and this disagreed with the findings of Gebrelibanos and Assen (2013), who reported low C/N ratio in GL. The rise of C/N ratio in GL of the study watershed might be due to the prevalence of higher undecomposed straw and lower mineralization (Landon, 1991).

CEC and exchangeable cations

High (> 40 $Cmol_c\ kg^{-1}$soil) (Landon, 1991) CEC in NFL of Wanka watershed most likely resulted from high SOM content of the soil as CEC is a function of SOM and clay fraction (Aghasi et al., 2011). The correlation coefficient results showed a significant ($p < 0.05$) positive association ($r = 0.46$) between SOM and CEC (data not shown). CEC also significantly increased in lower elevation than upper (0.01) and middle ($p < 0.05$) elevations as there was clay fraction increment in lower elevation.

The decline trend of exchangeable cations (Ca, Mg) in CL of the study watershed may be due to continuous cultivation coupled with limited application of organic fertilizers (e.g., less recycling of crop residues). As observed during the soil sample collection, farmers used crop residue mainly for animals feed instead of recycling it in the soil. In CL, there is a reduction of significant amount of soil nutrients with minimal return rate every year, and this subsequently diminishes SQ substantially (Duguma et al., 2010). The other possible reason may be losses of nutrients through leaching in CL that is accentuated by tillage as high leaching is associated with loses of exchangeable bases (Abegaz et al., 2016). This finding agreed with the finding of Wang et al. (2001) who reported that while soil under NFL with high total exchangeable bases had a better quality, soil in continuous CL had low total exchangeable bases and in turn poor quality in southern Tanzania. The divalent exchangeable cations (Ca^{2+} and Mg^{2+}) showed a decreasing trend with increasing elevation. Exchangeable Ca^{2+} and Mg^{2+} were lower in upper than the lower elevation significantly ($p < 0.01$). This is perhaps due to high leaching as rainfall increases with increasing elevation. In addition, lower cations in upper elevation of Wanka watershed may be associated with lower pH. Soils with low pH have low availability of Ca^{2+}, Mg^{2+} and phosphorus (Wang et al., 2001).

The overall DI values in Wanka watershed implies the existing land uses (CL and GL) are accelerating deterioration of SOM, TN and available phosphorus and increased soil compaction. Total DI of both CL (-21%) and GL (-24%) are rated as high negative DI (Wang et al., 2001). Similar findings were reported by several authors (Eyayu et al., 2009; Gui et al., 2009; Lemenih et al., 2005; Wang et al., 2001). High negative DI values is an indication of high SQ deterioration, which might have resulted from lack of appropriate soil management that maximize soil nutrient availability after the conversion of NFL to other LULC types (Schjønning et al., 2004; Fu et al., 2000).

Conclusion

The study disclosed that the impact of LULC and elevation variations is considerable on key SQ indicators. Declining SOM, TN, available phosphorus and increasing BD in CL of the study watershed signifies the impediment of infiltration and nutrient availability for crops. This could be attributed to soil management induced problems like over cultivation without appropriate soil management. This implies that prior emphasis needs to be given to CL, and hence, strategies that maximize SOM and reduce soil compaction like application organic fertilizers and minimum tillage should be emphasized. Elevation impact was also found significant on basic cations (Ca^{2+} and Mg^{2+}), CEC and pH. Their reduction in higher elevation of the study area implies the need for elevation specific land management interventions. Decline of pH value in the CL of upper elevation has an implication of reduction of microbial activities that increase SQ (reduction of microbial conversion of $NH^{+}4$ to nitrate (NO^{-}_{3}), and the decline of base cations and crop yield. Thus, lime application in the CL as well as soil and water conservation measures like terrace need to be encouraged in the upper elevation. Moreover, further investigation about the effect of variation in LULC and elevation on SQ indicators in the sub surface soil layer of the study area is required, as SQ is not entirely a reflection of surface layer.

Acknowledgements

The authors would like to acknowledge Adet Agricultural research centre, Ethiopia for the soil analysis. Our thanks also extend to the farmers of the study area for their cooperation in soil sample collection in their plots.

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Ekológia (Bratislava) Vol. 38, No. 2, p. 140–153, 2019
DOI:10.2478/eko-2019-0011

THE APPLICATION OF DIRECTIONAL UNIVARIATE STRUCTURE FUNCTIONS ANALYSIS FOR STUDYING THE SPATIAL ANISOTROPY OF ENVIRONMENTAL VARIABLES

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Abstract

Svidzinska D.: The application of directional univariate structure functions analysis for studying the spatial anisotropy of environmental variables. Ekológia (Bratislava), Vol. 38, No. 2, p. 140–153, 2019.

As anisotropy is a fundamental property of the real-world environmental spatial variables, the conventional omnidirectional variograms and correlograms do not provide means enough to characterise spatial dependence between observations. The purpose of this article is to introduce directional univariate structure functions analysis to explore and quantify the spatial anisotropy of environmental variables. Analysis of six environmental variables within three physical–geographical regions proved the leading role of relief for landscape differentiation; it also defined the size and extension of major landforms responsible for the organisation of spatial pattern. The arrangement of the vegetation patches demonstrated linkage with the major landforms. The other relief derivatives, being prone to noise and artefacts in the original data, showed a random-variable type of behaviour. In the lack of any particular spatially anisotropic structure, the results of the analysis can provide a clue about meaningful distances of interest at finer scales. The approach can also be an exploratory tool for discrete measurements to recognise the features of spatial continuity.

*Key words***:** autocorrelation, environmental variable, Kanivs'kiy Nature Reserve, semivariance, spatial analysis, spatial anisotropy.

Introduction

Most of the real-world environmental variables are inherently anisotropic, which means that spatial dependence between a variable's values is not the same for all geographic directions considered (Cressie, 1993; Legendre, Legendre, 2012; Rossi et al., 1992). It is important to account for the presence and features of this dependence to understand spatial patterns in the data (Legendre, Fortin, 1989; Rossi et al., 1992; Turner, Gardner, 2015), to catch the scale of data variability (Legendre, Legendre, 2012), to reveal links between variables (Rossi et al., 1992; Turner, Gardner, 2015) and to describe the main features of spatial anisotropy (Rossi et al., 1992). This information is routinely used to hypothesise on the nature and scale of a pattern-generating process (Legendre, Fortin, 1989; Turner, Gardner, 2015) and to develop sampling schemes (Cressie, 1993).

The tools to characterise spatial dependence between observations are the univariate structure functions of semivariance and autocorrelation (Cressie, 1993; Legendre, Legendre, 2012; Rossi et al., 1992). Both are calculated across a distance, whilst the semivariance is a measure of the variance and the autocorrelation is a measure of the correlation of a regionalised variable (Cressie, 1993; Legendre, Legendre, 2012). When used simultaneously, these functions provide insights on the existence of a spatial pattern in the data, degree of its spatial variability and critical distances at which significant similarities or dissimilarities are observed (Legendre, Fortin, 1989; Rossi et al., 1992; Turner, Gardner, 2015).

Traditionally, a single semivariance or autocorrelation value is calculated for each distance class, producing the so-called omnidirectional variograms and correlograms. But Legendre and Legendre (2012) argued that for spatial environmental variables, when anisotropy is the case, it is necessary to account for a directional change in the values of the univariate structure functions. Whilst the directional univariate structure functions analysis is recognised to be more appropriate for the spatially anisotropic real-world data, its practical implementation may be limited by a small number of irregularly spread observations (Legendre, Legendre, 2012) as well as the lack of ready-to-apply analytical and visualisation techniques (Legendre, Fortin, 1989; Rosenberg, 2000; Rossi et al., 1992).

Here, I describe how to apply the directional univariate structure functions analysis to the real-world environmental data. I examined the set of six raster environmental variables for the three sub-areas of different physical–geographical regions. Using the data, I illustrate how the results can identify and quantify complex spatial patterns and their scales. This, in turn, helped to recognise factors and/or processes responsible for landscape heterogeneity at different scales.

Material and methods

Study area

The study area of 1,528 km^2 covers the overall extent of the Kanivs'kyi Nature Reserve, Ukraine, and its surroundings, defined by a 2-km buffer zone (Copernicus Programme, 2015; Hansen, DeFries, 2007) (Fig. 1). As a part of the Dnieper River valley, the area encompasses three contrast physical–geographical regions (Marynych et al., 2003) (Fig. 2). Elevated erosionally dissected loess plains with gray podzolic soils under the secondary oak-hornbeam forests and arable lands on typical chernozems represent the first region. The second region is the fragment of a floodplain and alluvial terrace with meadow soils on alluvial deposits and soddy-podzolic soils on ancient alluvial deposits under meadow vegetation and pine forests, respectively. The third region within the left bank of the Dnieper includes the floodplain with soddy weakly-podzolic sandy soils under meadow vegetation and the first-third terraces with gray podzolic soils under the pine forests and typical chernozems under the arable lands.

The area harmoniously combines unique cultural and natural landscapes that has led to the consolidation and expansion of the existing protected areas and their recognition as the Key Biodiversity Area and Emerald sites (BirdLife International, 2018; Chorny, Chorna, 2013; European Environment Agency, 2017). Despite the importance of the area for the preservation of natural heritage and biodiversity, there is still the shortage of detailed information on the spatial pattern of its landscapes. In this respect, understanding of the main features of the spatial structure of environmental variables could provide a basis for field research, habitats inventory and landscape mapping necessary for the spatially precise data-driven decision making on nature conservation and land management.

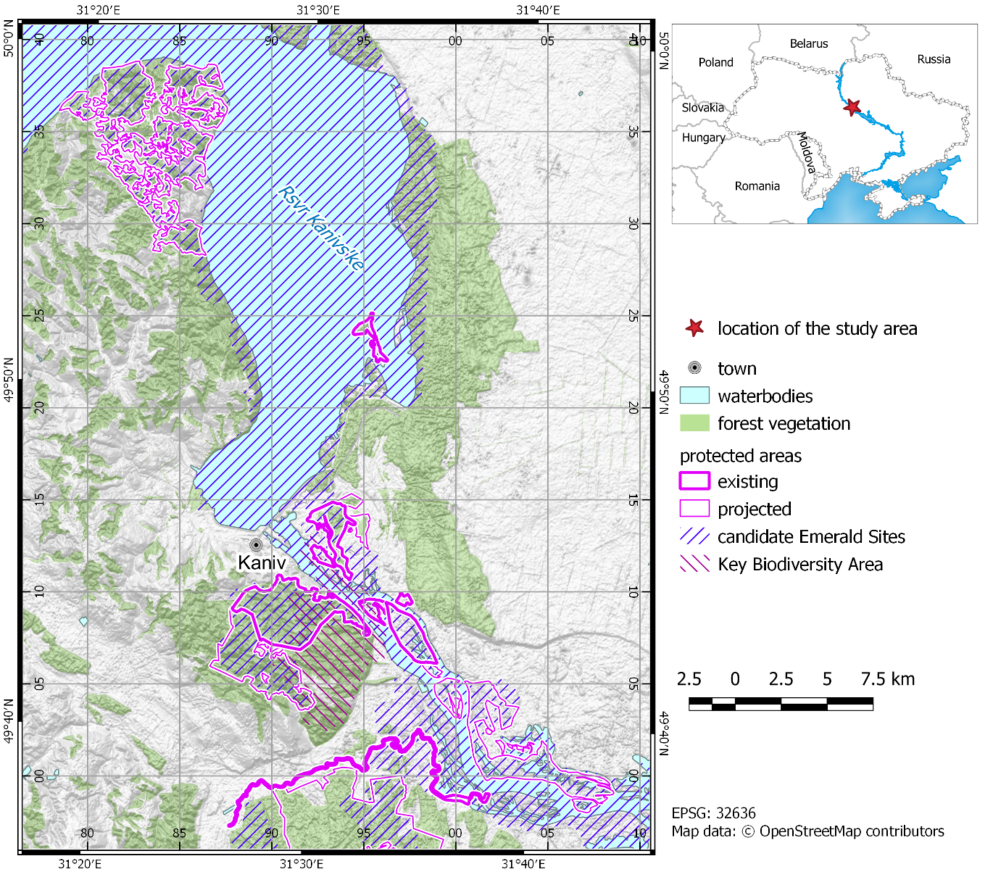

Fig. 1. Location and general view of the study area.

Materials

As landforms are an important factor of landscape heterogeneity (Swanson et al., 1988), terrain parameters composed a primary set of environmental variables. The Advanced Land Observing Satellite (ALOS) global digital surface model (DSM) 'ALOS World 3D – 30m' (AW3D30) v.2.1 released in April 2018 represented the terrain of the study area. The AW3D30 is a DSM data set with a horizontal resolution of 1 arc-second latitude and longitude mesh generated from the 5-m resolution DSM based on the images collected during 2006–2011 (Tadono et al., 2016). For the purpose of the analysis, the original DSM data were reprojected using bilinear interpolation from the geographic coordinate system WGS84 to the projected coordinate system WGS84/UTM zone 36N with the resulting spatial resolution of 30 m.

Multiple geomorphometric parameters have been developed and used to characterise the terrain variability (Hengl, Reuter, 2009; Wilson, Gallant, 2000). At the same time, Lecours et al. (2017) argued that more than 70% of environmental variability related to the relief is captured through the limited set of the six parameters, namely, local mean, slope, local standard deviation, relative difference to mean, easterness and northerness. I derived the suggested parameters from the AW3D30 DSM in the SAGA GIS (Conrad et al., 2015) that provides the algorithms recommended by Lecours et al. (2017).

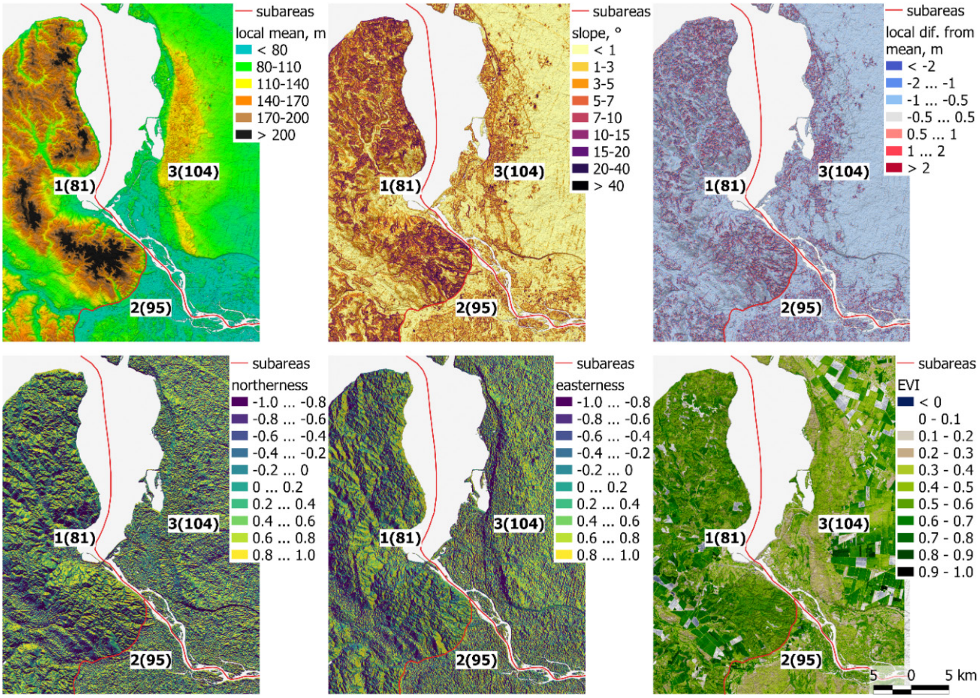

Fig. 2. Environmental variables selected for the analysis. Numbers of the sub-areas in brackets refer to the numbering scheme from Marynych et al. (2003).

The pattern of natural vegetation modified by the land-use practices is another important factor of landscape variegation. The continuous field variables of spectral vegetation indices can capture the short-term response of the vegetation to ecological and anthropogenic driving forces. For this purpose, I used the Enhanced Vegetation Index (EVI) with a spatial resolution of 10 m calculated from the Sentinel 2A Level 2A Bottom of Atmosphere (BOA) reflectance product of the image captured on 22 July 2017. The EVI was chosen instead of the commonly used Normalised Difference Vegetation Index because it incorporates adjustments for the canopy background and atmospheric effects, which increase its sensitivity to the vegetation signal (Huete et al., 2002).

Covariation is a common property of many environmental variables, especially of those derived from a single data source such as digital elevation model (DEM) (Graham, 2003; Lecours et al., 2017). To avoid possible data redundancy, I performed the correlation analysis to account for confounding spatial variables (Dutilleul et al., 1993; Osorio, Vallejos, 2014; R Core Team, 2017). Following the results of the correlation analysis, local standard deviation was excluded from the initial set of variables because of its strong (0.99) and significant ($p \leq 0.01$) correlation with slope. The final data set included 6 environmental variables (Fig. 2).

Methods

The analysis documented as a series of scripts for the R environment (R Core Team, 2017) and based on the packages EcoGenetics (Roser et al., 2017), geoR (Ribeiro Jr., Diggle, 2016), raster (Hijmans, 2016), rgdal (Bivand et al., 2017) and sp (Bivand et al., 2013; Pebesma, Bivand, 2005). The workflow included the following steps:

1. Import of a variable's raster layer to the computing environment and calculation of the default distance of interest as the one-third of the diagonal of the raster extent. This limitation is necessary to ensure that each lag contains a sufficient number of pairs of points to produce a reliable univariate structure function value (Legendre,

Legendre, 2012). Turner and Gardner (2015) recommended the overall linear extent of the data set to be at least twice the maximum distance (scale) to examine in order to encompass the process of a certain scale. In such a way, the distance limitation narrows down the scale of the analysis to the processes that reveal within the area of interest.

2. A random sampling of the raster by the user-defined number of points. The sample has to be limited because of computational requirements, and taking into account the distance limitation, usually 1000–3000 points are enough to produce reliable results. For the purpose of this analysis, in each case, I limited the sample size to 5000 points. The sample was exported to the ESRI Shapefile and stored as a separate data object for further analysis.

3. The calculation of the lag based on the maximum possible number of the pairs of points in the distance matrix by using the following formula:

$$N_{pairs} = \frac{n_{pixels} \times (n_{pixels} - 1)}{2}$$

where is the total number of the pairs of points in the distance matrix and is the total number of not-null pixels in the raster.

The definition of the possible number of classes and lag distance relies on Sturges' rule:

$$l = \frac{L}{1 + 3.322 \times log_{10}(N_{pairs})}$$

where is a distance lag and is the maximum distance defined in the first step. The denominator of the formula defines the number of distance classes. For the convenience, the lag value is rounded to be a multiple of 5.

On the basis of these values, the distance bins are defined by incrementing the lag by the number of classes from zero up to the maximum distance. All the results (the lag, number of classes, distance bins) are stored in intermediary data objects to be used in the following steps.

There is also a possibility for a user to manually define the lag and/or maximum distance of interest. But the simultaneous predefinition of both is not recommended because it deprives the analysis of its sense. The approach, when the lag and distance bins are based on the data properties, ensures that semivariance and autocorrelation values are calculated from the number of pairs of points that is large enough to produce reliable values. This is especially important with the limited sample size (Legendre and Legendre, 2012; Shaukat et al., 2016), which may be an issue for the sample-based analysis of the univariate structure functions of large data sets, such as rasters of continuous environmental variables.

4. Semivariance analysis using the parameters derived in the previous step (Cressie, 1993). The variograms are calculated for the nine directions from 0° to 180° with the increment of 22.5° and tolerance angle of 11.25°. In addition to these directions, an omnidirectional variogram is also calculated. For better control of the validity of the values, the minimal number of pairs of points for semivariance calculation is limited to 50 (Legendre, Legendre, 2012).

Because semivariance characterises the variables with different measurement units and levels of spatial variability, it is necessary to standardise its values for meaningful compatibility and interpretation. For this purpose, I applied the approach by Rossi et al. (1992) when the values of semivariance are divided by the overall sample variance. The results are stored as a CSV-file and include the semivariance values (both original and standardised) for bins' centres, number of pairs within each bin and their standard deviation.

5. Spatial autocorrelation analysis based on Moran's I coefficient (Rosenberg, 2000). The omnidirectional and nine-directional (from 0° to 180° with the increment of 22.5°) correlation coefficients are calculated for the defined distance bins. The results are stored as a CSV-file and include the autocorrelation values, number of pairs within each bin, mean distance between them and p-values calculated by permutations.

Visual analytical exploration of the results relies on two-dimensional or planimetric variograms and correlograms (Legendre, Fortin, 1989; Rosenberg, 2000; Rossi et al., 1992; Wickham, 2009). The resulting polar plot consists of two parts. The top part displays the change in semivariance (or autocorrelation and its significance) values in the space of the directions from 0° to 180°. As the plot is symmetric about its origin, the bottom 180–360° part would mirror the top one. But instead of mirroring the directional values, the bottom part is used to visualise the omnidirectional values (to be identical in all directions). The combination of the directional and omnidirectional values in a single plot simplifies the exploration and understanding of the presence and features of the spatial anisotropy in the data.

Results

The data-derived parameters calculated from the rasters by the sub-areas enable the exploration of spatial dependencies over the scales within the range from zero to the maximum distance with the grain of the lag (Table 1).

T a b l e 1. The parameters of the univariate structure functions analysis.

Variable	Lag, m	Number of distance classes	Maximum distance, m
Sub-area 1			
DEM derivatives	415	37	15 355
EVI	350	44	15 400
Sub-area 2			
DEM derivatives	270	34	9 180
EVI	225	39	8 775
Sub-area 3			
DEM derivatives	410	38	15 580
EVI	350	45	15 750

Sub-area 1

The local mean variable demonstrates a well-identifiable spatially anisotropic structure (Fig. 3). According to the variogram, the main direction of the data variability goes along the 90–270°[1] axis with the contours elongating along 135–315° and shrinking along 22.5–202.5°. Two primary semivariance peaks of 1.3 and 1.2 occur at a distance of 7 km (the 22.5–202.5° axis) and 11 km (the 157.5–337.5° axis), respectively. The overall data variability reaches its maximum of 2.1 at a distance of 13 km. The correlogram demonstrates the presence of significant positive autocorrelation up to 5 km with the elongation of the contours up to 8 km in the 146.25–326.25° direction. An additional contour of zero autocorrelations appears at a distance of 12 km.

The comparison of plots with the map of local mean suggests the presence of a two-level hierarchy in the arrangement of relief spatial structures. The variability of a finer scale is present at a distance of 5–7 km. It refers to the elevated ranges of the prevailing NW–SE orientation resulted from the dissection of hills by major elements of the erosion network. The broad-scale differences are the most clearly pronounced along the N–S direction and attribute to the landforms of a linear size of 12–13 km, which supposed to be the hills and lowlands referring to the morphostructures formed by horsts and grabens.

Some signs of the fine-scale structure of the slope variable at a distance of less than 1 km present at the variogram and correlogram. The overall variability of the values pronounces in the 90–270° direction and reaches its maximum of 1.7 at a distance of 15 km. The autocorrelation

[1] Here and thereafter for convenience, the directional axes are given for the whole circle of 0–360° because, owing to the symmetry, the bottom (180–360°) part of the plot would mirror the top (0–180°) part.

values change most rapidly along the 0–180° axis with the elongation of the contour of zero correlations in the latitudinal direction from 7 km to the distance exceeding the extent of the analysis.

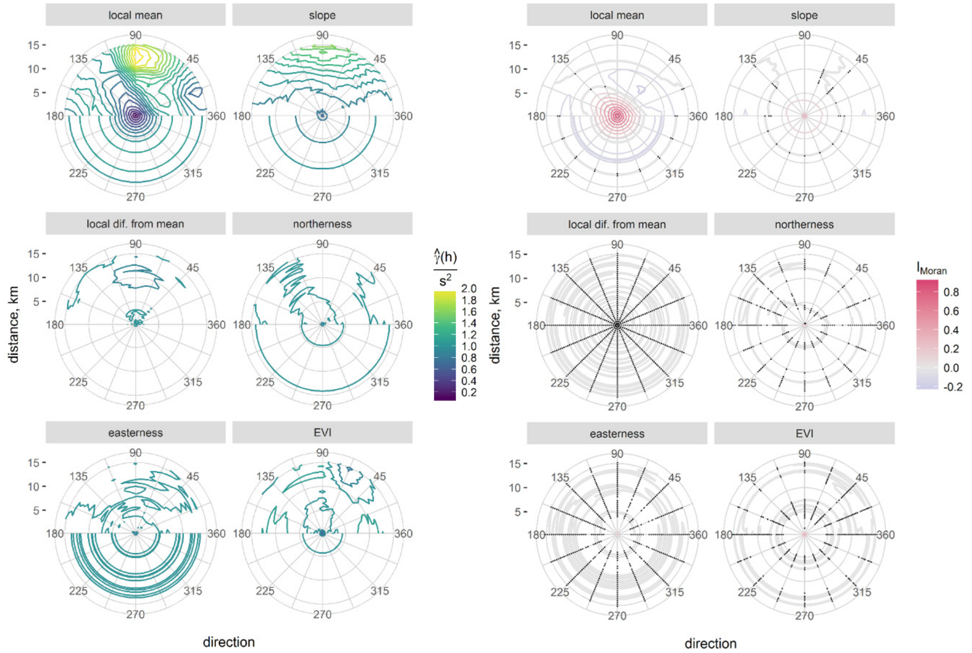

Fig. 3. The two-dimensional planimetric variograms (a) and correlograms (b) for sub-area 1. Black dots mark insignificant (p > 0.01) autocorrelation values.

The variogram and correlogram of the local difference from mean variable demonstrate the absence of any spatially structured anisotropy with the semivariance values fluctuating around one and zero correlations of low significance.

According to the variogram, the spatial anisotropy for the northerness variable is not evident, as all semivariance values are around 1. But the correlogram demonstrates weak positive significant correlations up to a distance of 3 km with the elongation of the contours in the 146.25–326.25° direction up to a distance of 5 km. This is because sub-area 1 represents the elevated erosionally dissected landforms with pronounced slopes. As it was shown by the analysis of the local mean variable, the ranges of these landforms are mostly oriented along the NW–SE direction. These features are captured and reflected in the northerness spatial variability.

Similarly, for the easterness variable, there is no clear pattern on the variogram, but the correlogram demonstrates weak positive significant correlations up to a distance of 3.5 km (with the slight elongation of the contours along 90–270°). This is because easterness, being a direction-dependent variable, most clearly pronounces for the landforms oriented along the N–S direction.

The features of the EVI spatial structure are not obvious from the variogram. According to the correlogram, weak positive significant correlations persist up to 3–5 km with minor fluctuations, depending on the direction. This distance refers to the major size of the vegetation patches. The scale of vegetation pattern variability is, to some extent, linked to the local mean variable that may be explained by the landforms-driven distribution of land-use pattern in the area. As a result, the large patches of the vegetation (semi-natural or agricultural) coincide in their size with the size of the most prominent landforms.

Sub-area 2

The local mean variogram demonstrates the presence of a spatially anisotropic structure with the broad-scale trend of the data variability oriented along the 22.5–202.5° direction and its possible maximum extended beyond the distance of interest (Fig. 4). On a finer scale, the data reach the plateau of 1 at a distance of 4 km with the contours elongated in the 101.25–281.25° direction. The correlogram demonstrates the most rapid change in values in the 67.5–247.5° direction. The contour of zero correlations is located at a distance of 4.5 km with fluctuations of ±0.5 km depending on the direction. The second contour of zero correlations appears along the 135–315° direction at a distance of 8 km.

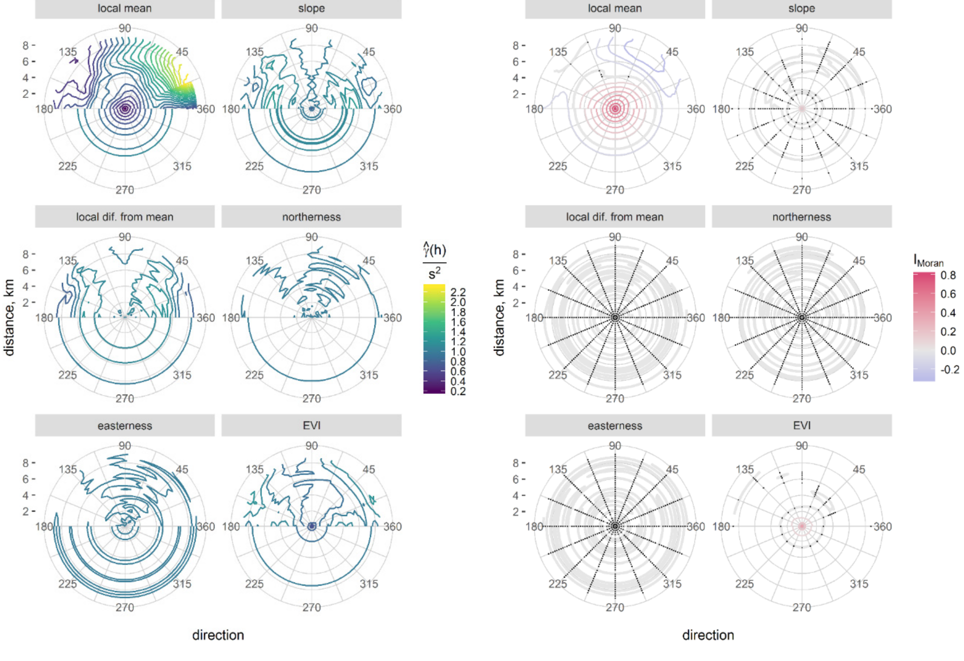

Fig. 4. The two-dimensional planimetric variograms (a) and correlograms (b) for sub-area 2. Black dots mark insignificant (p > 0.01) autocorrelation values.

According to the variogram, the major trend of the local mean variability oriented perpendicularly to the river valley along the ENE–WSW direction. As the trend does not reach its plateau within the distance of interest, it may identify the influence of the landform of a broader spatial scale than the area of interest (the right-bank low terrace). On a larger scale related to the landforms of a linear size of 3–4 km, a single structural element is present. It represents an elevation in the central part of the sub-area with the slight expansion in the NNW–SSE direction and conveys not the relief but rather a perforated fragment of pine forest vegetation fixed in the AW3D30 data as an artefact. As the vegetation artefacts have not been removed from the DSM, they become especially evident in the flat areas with no prominent relief features (which is the case for sub-area 2).

The slope variogram demonstrates symmetry about the 90–270° direction. Two variability maximums of 1.4 registered at a distance of 5 km on both the sides of the 0–180° axis. The correlogram demonstrates the presence of weak positive significant correlations up to a distance of 2 km, marked by the contour of zero correlations. This means that slope variability is symmetric in respect to N–S direction with the most rapid changes along the NW–SE and ENE–WSW diagonals and the overall extent of data similarity of 2 km. These features refer to the geometry and linear size of a forest vegetation-related artefact.

The variogram of the local difference from mean variable demonstrates some subtle features of a structured symmetry about 90–270° direction with the moderate peaks of 1.2 at a distance of 5 km along the 22.5–202.5° and 157.5–337.5° directions. It can be assumed that overall data variability related to a general convexity–concavity pattern of the surface, which, in turn, may be attributed to the vegetation artefact fixed in the DEM. But the correlogram with zero values of low significance shows no evidence of any particular spatial structure.

The univariate structure functions of the northerness and easterness variables fluctuate around the semivariance values of one and zero correlations of low significance. This type of functions' behaviour rejects the presence of any spatial structure for these variables.

According to the EVI variogram, there is no evidence of any clearly pronounced spatial structure, because most of the values do not exceed 1 or fluctuate around it. However, two moderate peaks of 1.2 occur at a distance of 8 km in the 22.5–202.5° and 157.5–337.5°directions. This pattern is linked to the variograms of the slope and local difference from mean variables and reflects the shape and geometry of the vegetation artefact. The correlogram demonstrates almost spatially isotropic weak positive significant correlations up to a distance of 3 km, marked by the contour of zero correlations. This distance refers to the generalised linear size of vegetation patches in the area.

Sub-area 3

The local mean variogram reaches its plateau at a distance of 9 km along the 90–270° direction (Fig. 5). The directions of maximum variability of 1.5 are oriented along the 45–225° and 157.5–337.5° diagonals. The local mean correlogram demonstrates the presence of positive significant correlations up to a distance of 10 km with the general latitudinal elongation of the contours. The fragments of the secondary contours of zero correlations appear in the longitudinal direction at a distance of 13 km.

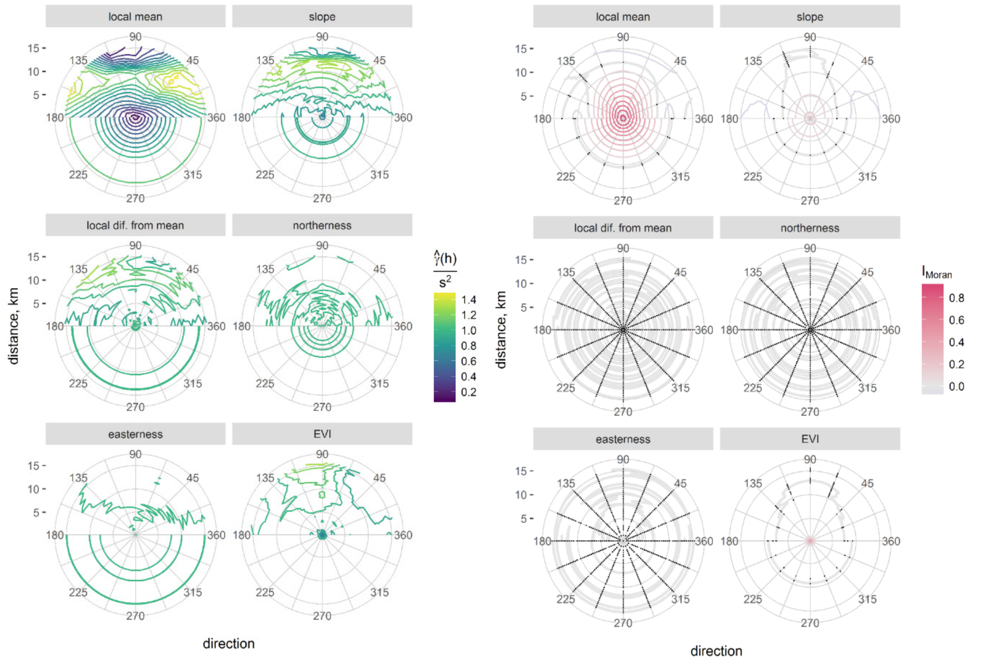

Fig. 5. The two-dimensional planimetric variograms (a) and correlograms (b) for sub-area 3. Black dots mark insignificant (p > 0.01) autocorrelation values.

According to the variogram and correlogram, within sub-area 3, the relief of the area demonstrates low variability with the main spatial structures latitudinally oriented along the river valley and the most rapid changes occurring along the NE–SW and WNW–ESE directions. Comparing this information with the map, it can be concluded that major landforms responsible for the differentiation are the floodplain and first, second and the fragment of the third terraces of the Dnieper clearly identifiable in the relief.

According to the slope variogram, the main direction of the data variability orients along the 135–315° direction and reaches its maximum of 1.4 at a distance of 15 km. The overall plateau of the variogram is most clearly pronounced in the latitudinal direction at a distance of 12 km. At the correlogram, the contour of zero correlations elongates latitudinally and passes at distances from 6 to 13 km depending on the direction. Within sub-area 3, the slope variable is most clearly linked to the variable of local mean. This is because the main changes in the slope values are strictly related to the curbs and ledges of the river terraces.

The variogram of local difference from mean demonstrates some resemblance of its contours pattern to the slope variogram. The trend of the maximum variability lies along the 135–315°(NW–SE) direction. A possible explanation for this is that the distribution of noise and artefacts registered by local difference from mean in this sub-area related to the distribution pattern of vegetation patches (forest and agricultural), which in turn coincide with the major landforms. At the same time, the correlogram does not reveal any pattern and presents close to zero correlation values of low significance.

The semivariance values of northerness and easterness fluctuate around 1; correlograms demonstrate zero correlation values of low significance. The univariate structure functions do not confirm the presence of any spatial structure within sub-area 3 for the variables of northerness and easterness.

The EVI variogram demonstrates the major trend of the data variability oriented along the 90–270° direction and reaches its maximum of 1.3 at a distance of 15 km with a possible plateau probably exceeding the distance of interest. At the correlogram, the contour of zero correlations elongates latitudinally and passes at distances from 7 to 13 km depending on the direction. For this sub-area, the driving forces of vegetation pattern are tracked at two levels. At the first level, there is a fine-scale variability within a distance of 1 km related to the internal heterogeneity of vegetation patches such as individual agricultural fields, meadows and forests. At the second level, these are broad-scale patterns explained by overall land-use differences: the areas mostly dominated by the agricultural vegetation as opposed to the areas under (semi)natural vegetation. At this level, the EVI plots demonstrate some resemblance to the plots of local mean, slope and local difference from mean. This is because the distribution of land-use pattern in the area coincides with the major landforms. The floodplain and first terrace are covered with meadows, the second terrace is under agricultural land use and pine forest grows on the fragments of the third terrace.

Discussion

At a given scale of the analysis, not all environmental variables turned out to be equally responsible for the presence of a spatial pattern and informative for its explanation within the study area. The local mean variable within each sub-area demonstrates a clearly identifiable spatial structure with the obvious features of spatial anisotropy. Comparison of the distances and directions of the maximum semivariance and zero autocorrelations with the linear size and orientation of the landforms allowed assuming the scale of the landforms, which define the major features of the spatial structure of the area as well as their linkages to the other variables. For sub-area 1, these are the NW–SE oriented elevated ranges with a length of 5–7 km. For sub-area 3, these are latitudinally oriented structural elements of the river valley (the floodplain and terraces). In both the cases, the vegetation pattern demonstrates linkages with the relief because the spatial distribution of the land use tends to follow the major landforms.

The other DEM derivatives are less informative. The pattern of the slope variable spatial anisotropy usually inherits small-scale features of the local mean variable. The variable of local difference from mean for all sub-areas, excluding sub-area 2, demonstrates a type of behaviour close to a random variable characterised by the flat variograms (the values fluctuating around 1) and zero correlations of low significance ($p > 0.01$). Similarly, the variables of northerness and easterness behave randomly, excluding sub-area 1.

The random type of behaviour symbolises the absence of any detectable spatial trends or patterns and can be attributed to two reasons. First, the structure may reveal itself at a much larger scale being registered at the scale of the analysis as a random noise. Second, the derivatives of an initial surface are prone to be affected by the noise represented by speckle and artefacts in the original data, which make the spatial structure less detectable.

In addition, in flat areas, vegetation tends to be registered in the DEM as artefacts. This primarily affects the resulting values of slope and local difference from mean, which characterise the shape of a surface (steepness, convexity–concavity). Because this shape reflects a vegetation pattern, the variograms and correlograms of slope, local difference from mean and EVI may demonstrate resemblance, as within sub-areas 2 and 3.

Nevertheless, even if the variables demonstrate a weakly detectable structure, the results of the analysis usually suggest the potential distances of interests at a larger scale, similar to that in the case of EVI for all the sub-areas or northerness/easterness within sub-area 1. This prevents arbitrary decisions and multiple tests and trials for choosing the parameters for a further detailed exploration.

Variograms and correlograms of a single variable usually demonstrate the overall resemblance accompanied by some discrepancies in the directions and characteristic distances of the contours' expansion. These discrepancies could make interpretation more difficult. Rossi et al. (1992) argued that it is necessary to jointly interpret variograms and correlograms because they differently account for local variability and thus highlight different aspects of the data. Variograms do not filter out lag means and variances, so they reveal a lag-to-lag local variability and underline the dissimilarity of values. Correlograms, on the contrary, account for regional patterns because of filtering out local variability and accentuate similarity in values. Also, because variograms do not filter out a large-scale variability, they tend to overestimate characteristic scales of spatial anisotropy. These differences partially explain the dissimilarities of variograms against correlograms.

Another part of the explanation attributed to the differences between the algorithms in definition and calculation of the direction. Variograms are calculated with the ±11.25° directional tolerance, which slightly blurs the resulting values (Cressie, 1993; Ribeiro Jr., Diggle, 2016). The calculation of correlograms involves the weighing of distance matrix elements based on their association with a certain fixed direction (Rosenberg, 2000; Roser et al., 2017). These features of the algorithms may also add up to the inconsistencies in patterns.

Conclusion

Comparing to conventional single-dimensional omnidirectional univariate structure functions, directional functions conveyed through an appropriate visualisation identify main features of spatial anisotropy of environmental variables. Joint exploration of directional correlograms and variograms allows for the confirmation or rejection of the existence of a spatial pattern and, in the case of its presence, to describe its spatial anisotropy; to quantify the data variability range, the scale(s) of maximum variances and zero correlations; and to check for the links between variables. These characteristics help to identify main features of spatial variability and its scales, to investigate the dissimilarities in the spatial patterns related to the processes of different scale and to hypothesise on the underlying factors and/or processes responsible for landscape heterogeneity at a certain scale.

In the case of absence of clearly identifiable spatial anisotropy, the analysis based on the data-derived parameters should be considered as an exploratory technique. Its results can be applied to guide further investigation and to identify informative scales and distances of potential interest.

For DEM derivatives, which are prone to be distorted by noise and artefacts, the quality of the data affected the ability to identify large-scale spatial patterns. This question requires further investigation in the context of both DEM preprocessing techniques (Gallant et al., Dowling, 2012) and the potential to use newly available more detailed sources of elevation data (Grohmann, 2018).

Acknowledgements

I thank Professor Mykhailo Grodzinskyi for comments that improved an earlier version of this manuscript. I also thank Copernicus Research and User Support (RUS) Service for providing the computing environment to process and analyse the data.

Supplementary material

The data sets analysed and generated during the current study, codes and full-size figures associated with this article are available in the Open Science Framework repository at https://osf.io/3hfq6.

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Ekológia (Bratislava) Vol. 38, No. 2, p. 154–165, 2019
DOI:10.2478/eko-2019-0012

PAYMENTS FOR FOREST ECOSYSTEM SERVICES ACROSS EUROPE – MAIN APPROACHES AND EXAMPLES FROM SLOVAKIA

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Abstract

Sarvašová Z., Báliková K., Dobšinská Z., Štěrbová M., Šálka J.: Payments for forest ecosystem services across Europe – main approaches and examples from Slovakia. Ekológia (Bratislava), Vol. 38, No. 2, p. 154–165, 2019.

Payments for ecosystem services (PES) are flexible, financial mechanisms for utilisation of available finances for environmental improvement. Payments for forest ecosystem services (PFES) have gained increasing policy acceptance at national and international levels. However, evidence about their implementation is limited and rather mixed. PES design is a complex task. There are a number of PES design features that need careful understanding of the specific ecological and socio-economic context. The aim of this article is to analyse main approaches to PFES and types of PES schemes or financial arrangements with the emphasis on three basic schemes: (i) public schemes or government-financed PES (Pigouvian type), (ii) private schemes or user-financed PES (Coasean type) and (iii) public–private schemes (a mixed type). The empirical part is based on the review of PES schemes implemented in different Forest Europe signatory countries. The main features of PES schemes are described on chosen examples from Slovakia.

Key words: financial mechanism, forest functions, payments schemes design.

Introduction

Three international classification systems are available to classify ecosystem services (ES): Millennium Ecosystem Assessment (MEA), The Economic of Ecosystems and Biodiversity (TEEB) and Common International Classification of Ecosystem Services (CICES). In essence, they relate, to a large extent, to each other; all three include provisioning, regulating and cultural services (Maes, 2013). MEA (2005) also distinguishes supporting ecosystem services that are necessary for the production of all other ecosystem services. Forest ecosystems provide an array of benefits, including protection of soil and water resources, habitat for fish and wildlife, timber and wood fibre, aesthetically pleasing landscapes and the storage of carbon that can help mitigate global warming (Nasi et al., 2002; Kilgore et al., 2017). As ES are mostly public goods, there is usually no market for them

(Pagiola et al., 2002) that has implications for forest managers as well as for policy makers. If a service has no market, it has no market price (i.e. the price is zero) and the forest owner will not consider the provision of this service in the same way as he or she would for a market good like timber. However, this non-market good or service has a value for the forest owner and for society (Garcia et al., 2018). Provisioning services are equivalents of forest production functions and include production of wood, game and other market products. These goods produced by forest ecosystems can be classified as private goods for which a market mechanism and a price operate as indicators of the limitation of a private farm (Bösch et al., 2018; Nasi et al., 2002; Mavsar et al., 2008). Cultural and supporting ecosystem services are equivalents of non-productive functions of forests and are considered to be public services with open access and inaccessibility from consumption (Table 1). As a result, these services do not have the manufacturer's proprietary rights, the ambiguous claim structure and unfair transaction costs (Sternberg, 1996). As no one is the owner or has rights to these services, and others cannot be excluded from their exploitation or benefits, there is no incentive for recipients to provide ecosystem services in a sustainable way (Daily et al., 2000).

T a b l e 1. Approaches to FES from Slovak law to worldwide classification.

<table>
<tr><th colspan="3">Functions of Forests (Act About Forests no. 326/2005 Coll.)</th><th>FES (MAES, 2013)</th><th>FES (MA, 2005)</th></tr>
<tr><td colspan="3">Productive (wood, hunting, non-wooden products)</td><td>Provisioning</td><td rowspan="10">Supporting</td></tr>
<tr><td rowspan="9">Non-productive</td><td rowspan="3">Ecological</td><td>Soil protection</td><td rowspan="6">Regulating/sustaining</td></tr>
<tr><td>Hydric-water management</td></tr>
<tr><td>Climatic</td></tr>
<tr><td rowspan="6">Social</td><td>Health</td></tr>
<tr><td>Nature protection</td></tr>
<tr><td>Water protection</td></tr>
<tr><td>Recreational</td><td rowspan="3">Cultural</td></tr>
<tr><td>Cultural</td></tr>
<tr><td>Educational</td></tr>
</table>

A major challenge regarding the delivery of forest ecosystem services (FES) is that many of the provided services are not tradable on the markets, making it difficult to observe their values directly (Forest Europe, 2014; Viszlai et al., 2016). Therefore, information and assessments of forest functions and ecosystems services is important for the design and implementation of related policies and implementation of effective sustainable forest management at the European level (Maes et al., 2013, 2014). Financial instruments are designed to modify behaviour by encouraging private individuals, organisations and businesses to actively participate in supporting ecosystem services (Raitanen et al., 2013). Conceptualising the schemes for the payments for ecosystem services (PES) is becoming an increasingly popular way to manage ecosystems using market-based incentives (Farley, Constanza 2010; Gómez-Baggethun, Muradian, 2015; Prokofieva, 2016). The core idea of PES is simple; users or beneficiaries of these services (Prokofieva, 2016) pay landowners or forest managers for the provision of certain ecosystem services or for a particular forest management strategy for generating the desired ES. Recently, a number of studies have been developed across Europe to map operation of PES schemes (Bösch et al., 2018; Smith et al., 2017; OECD, 2010; IUCN, 2009; UNECE, 2005, 2014). We describe the existing approaches to payments for forest ecosystem services (PFES) schemes with examples from Europe.

The aim of the article is to analyse main approaches to payments for FES and existing PFES schemes in Europe, with the emphasis on examples from Slovakia.

Characteristics of PES schemes

PES are flexible, financial mechanisms for utilisation of available finances for environmental improvement. We can also characterise them as a reward for ensuring positive externalities (Daily, 1997) and their internalisation (Farley, Costanza, 2010; Garcia et al., 2018). Wunder (2015) described PES as voluntary transactions between service users and service providers that are conditional on agreed rules of natural resource management for generating offsite services. He also critically addressed existing definitions of PES, discussed the distinguishing features of PES that differentiate them from other economic incentives and derived a revised definition of PES. Most of the literature refers to PES as a market-based or a market-like mechanism and follows the criteria of Wunder (2005). PES is defined as a scheme that follows the following conditions:

a. it is a voluntary transaction,
b. there is a well-defined environmental service (or a land use likely to secure that service),
c. there is a minimum one service buyer,
d. there is a minimum one service provider who acts as a seller,
e. it is paid if and only if the service provider secures service provision (conditionality).

Understanding how PES mechanisms work in theory and in practice, and knowing their limitations, is crucial for exploiting their full potential as a policy tool for solving complex environmental problems we are confronted with (Prokofieva, 2016). The mechanism of the PES scheme is based on the amount of the payment as shown in Figure 1.

To ensure that PES schemes are successful, it is important to achieve a win–win situation for both sellers and buyers. The payment offered to forest owners or forest managers must exceed the additional benefit they would receive from the alternative forest use (or they would not change their behaviour) and must be less than the value of the benefit to FES users (or users would not be willing to pay for it) (Engel et al., 2008). The minimum PES should be generally expected to cover at least any (private) returns forgone as a result of reduced timber production. The theoretical maximum payment would represent the cumulative value of additional ecosystem service benefits that would accrue to the buyers. Many of these benefits are still hard to quantify (Smith et al., 2013).

A critical issue of PES schemes concerns the main actors involved in them. According to Engel et al. (2008), the actors are:

- **Buyers** – actual beneficiaries of an ES or 'others' (typically the government, a private company, association, or nongovernmental organisations [NGOs] and international agencies) acting on behalf of the users of the ES.
- **Providers** – are those actors (land and resource managers, local communities, farmers) who are in a position to safeguard the delivery of the ES and act as sellers.

In many PES schemes, groups of other actors have also been recognised. Governmental and NGOs play an important role in many PES schemes (e.g. Vatn, 2010; Huber-Stearns et al., 2013; Smith et al., 2013). They might be:

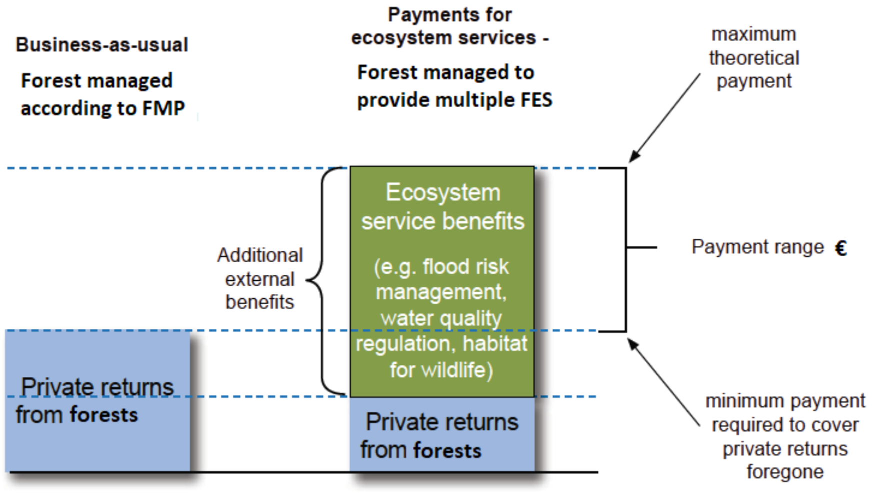

Fig. 1. PES mechanism (adapted from Smith et al., 2013).

- **Intermediaries** – are those who can serve as agents linking buyers and sellers and can help with scheme design and implementation.
- **Knowledge providers** – include resource management experts, valuation specialists, land use planners, regulators and business and legal advisors who can provide knowledge essential to scheme development.

A PES scheme can focus on more than one ecosystem service provision. The services being sold are then described as having been 'packed'. Ecosystem services can be packaged in three distinct ways (Fig. 2; Smith et al., 2013):

- **Bundling**: a single buyer, or a consortium of buyers, pays for the full package of ecosystem services that arise from the same parcel of land or body of water, for example, an agro-environment scheme funded by the government on behalf of the wider public.
- **Layering**: multiple buyers pay separately for the ecosystem services that arise from the same parcel of land or body of water; layering is also sometimes referred to as 'stacking'. For example, an area of peatland is restored and yields a range of saleable ecosystem service benefits. The carbon sequestration benefits are purchased by a business, the water quality benefits by a water utility, the flood risk management benefits by the government on behalf of downstream communities and the biodiversity benefits by a wildlife charity on behalf of its membership.
- **Piggy-backing**: in this case, not all of the ecosystem services generated from a single parcel of land or body of water are sold to buyers. Instead, a single service (or possibly several services) is sold as an umbrella service, whilst the benefits provided by other services accrue to users free of charge (i.e. the beneficiaries 'free ride'). For example, a business pays an upstream land manager for riparian restoration work to reduce the downstream flood risk to its bankside facilities. These improvements simultaneously improve water quality, enhance recreational

values and provide habitat for wildlife. However, no buyers are found for these additional services, and the benefits they provide to end users are received at no cost.

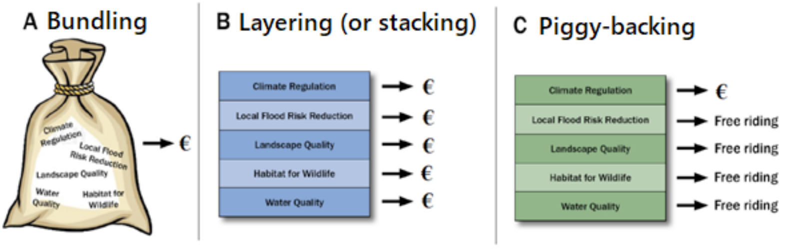

Fig. 2. Approaches to packaging ecosystem services (source: Smith et al., 2013).

PFES scheme types

A number of alternative approaches to PFES have been designed. According to the financial arrangements, PFES can be divided into three basic schemes (Schomers, Mantzdorf, 2013; Mantzdorf et al., 2013; Mavsar et al., 2008):

- **Public schemes** or Government-financed PES – in these, a public body, such as a municipality, a national or a local government, is the primary buyer of the ecosystem service, generally a land use or management practice is the general interest whilst also benefiting local concerns. Those buyers act on behalf of ecosystem services users – citizens or general public are the service users (Pigouvian-type).
- **Private schemes** or User-financed PES – privately owned bodies (such as companies, cooperatives or private individuals) compensate a private landowner for the maintenance of an ecosystem service. Buyers are the actual users of ecosystem services (Coasean type).
- **Public–private schemes** – a combination of public and private schemes. In these, the seller is a private entity, whilst the buyer (or one of the principal buyers) is also a private individual but represented by a public body. The PES contract is usually administered by a third-party PES-management entity.

According to the five PES criteria (Wunder, 2005), the PES schemes can be divided into another three groups (Zandersen et al., 2009):

- **PES core schemes** – only schemes that strictly follow the five main criteria – a voluntary transaction between a minimum one buyer and a minimum one seller of a well-defined ES and with a strong conditionality attached.
- **PES-like schemes** – incentives comply with only some of the five requirements. For example, some programmes may not have buyers paying voluntarily for the service or other programmes may be characterised by a low conditionality or a weak additionality.
- **Other economic incentives** – a range of economic incentives as PES, where payments are made to achieve higher levels of ES streams in different contexts.

Methodology

A document analysis of relevant primary and secondary sources was used to describe PFES and their characteristics. The main classification is based on five Wunder's (2005) criteria for PES schemes. According to them, we searched for specific examples and cases of public, private and public–private PFES schemes across Europe and the Slovak Republic. The description of selected European examples was based on the analysis of available information about PFES schemes from the following PES databases:

- UNECE-FAO database of PES case studies,
- European PES repository of Case Studies on PES-for-W,
- Forest Europe Web Portal on Forest Ecosystem Services,
- Ecosystem Marketplace.

For the purpose of this article, the UNECE list of PFES examples and Ecosystem Marketplace database were used as the main information sources. The interactive Ecosystem Markets Map displays information about projects that conserve, restore or support sustainable management of ecosystem services through ecosystem markets and market-based mechanisms. Examples from Slovakia were described based on the available information and the literature review, supported by the personal interviews with actors presented as ES providers. The individual examples were further described in terms of the actors involved in PES schemes, the supported ecosystem services and the packaging of the ecosystem services. The results of the comparison of the PFES schemes were elaborated using Wunder's (2005) PES criteria. All examples were used to support the existence of described theoretical approaches of PFES in Europe and Slovakia.

Results and discussion

PFES received a lot of academic attention in the past years (Schomers, Matzdrof, 2013; Prokofieva, 2016). Different PFES schemes have been developed across Europe to address problems with ensuring the provision of FES, nature conservation, biodiversity, water quality and other environmental concerns. A widely accepted policy strategy is to implement direct PES to increase the incentives of forest owners, public or private for providing such services.

From the point of the supported services, we divide PFES schemes into payments for forest services (PFS) and/or biodiversity (BD), payments for watershed services (PWS) and water quality improvement (UNECE, 2014). The Ecosystem Market Place Map uses different filter for distinguishing between the projects, that aim to support the ecosystem services, that generate: (i) the carbon offsets; (ii) the wetland and stream offsets; (iii) habitat offsets and (iiii) investments in watershed health. The Market Place database shows the total number of 137 PES projects across Europe, of which 22 focus on providing/conserving terrestrial carbon, 49 focus on providing/conserving species and habitats and 66 provide/conserve watersheds. There is no project providing or conserving wetlands (Ecosystem Marketplace, 2018). The UNECE list reveals another 48 schemes across Europe. Those databases do not contain all projects related to forests and are open for suggestions. Most PFES schemes focus on watersheds (UNECE, 2014; Ecosystem Marketplace, 2018).

Most of the PES schemes in the European Union are public–private ones (Ecosystem Marketplace, 2018; UNECE, 2014). Mixed schemes are based on different bilateral agreements, collective fund actions or compensatory mitigation (Ecosystem Marketplace, 2018). At present, the Ecosystem Marketplace and UNECE identify approximately 47 public PFES schemes at the European level. The issue of PES has reached the political agenda of the European Union, mainly through national rural development programmes (Kati et al., 2015; Sarvašová et al., 2013).

In Slovakia, public schemes are predominantly used to ensure FES provision. We identified three types of public PFES schemes: (1) forest land tax relief for protective and special purpose forests, (2) refunds for the restriction of ownership rights and (3) forestry support for non-productive forest functions (Table 2). Private schemes are very rare in Slovakia. Besides these national schemes, the Rural Development Programme also contains measures that can be considered as PFES, namely, Natura 2000 payments.

Most European countries have used a variety of incentives to encourage ecosystem services of forests (Mercer, 2004). Tax incentives include reduced or differed property, estate and inheritance taxes, favourable tax credits and deductions, favourable capital gains treatment of timber income as well as incentives linked to specific forestry practices such as wildlife protection, recreation and reforestation (Ma et al., 2014). The forest land tax relief for protective and special purpose forests in Slovakia represents the support for all services that are part of the forest ecosystem on the specific forestland. As Mercer (2004) pointed out, the protective role of forest ecosystems provides public goods (e.g. biodiversity, carbon sequestration, wildlife habitat, recreation, tourism), whose production may or may not conflict with private goods produced from the ecosystem such as timber, and tax incentives are way how to support the forest owners. A similar example can be found in France, where numerous fiscal mechanisms apply to protected areas, including land tax exemptions in Natura 2000 areas and land revenue tax reductions for expenses for the preservation or restoration of protected areas (IUCN 2009). In the case of this instrument, however, mainly the 'piggy-backing' principle is applied. The land tax relief is granted for a particular case of support for non-productive forest functions based on subcategories of special purpose forests and protective forests, although the other services are 'free riders'. Because the tax relief is not a direct payment and the principle of voluntariness or conditionality is not adhered, we can consider it as other economic incentives supporting FES. The fulfilment of the irreplaceable functions of forests and their preservation is also supported in the European countries by the environmental taxes (Schlegelmilch, 2002), from which governments create funds to support ecosystem services (Miceikiene, Butvilaite, 2015).

In the water sector, public schemes usually target services to secure supply (quality and quantity), flood protection and erosion control usually by the provision of financial incentives to encourage more sustainable land use (UNECE, 2014). The example from Slovakia is a private PFES water scheme between the Military Forests and Estates of the Slovak Republic (MFE), owned by the state, but more or less act as private company and local water management and supply company (Podtatranská vodárenská prevádzková spoločnosť, a.s.). A significant part of the territory of MFE includes important water areas classified into the first to third degrees of water protection. Many watercourses are sources of drinking water. In some places, water payment schemes are introduced to provide drinking water for local use (e.g. Branch office of the State Military Forest Enterprise in Kežmarok). The payment scheme for water is a long-term business relationship, lasting for more than 10 years. Contracts and prices are negotiated with the approval of the Regulatory Office for Network Industries of the Slovak Republic for 3 years. In these areas, the MFE provides water management in accordance with the area needs and environmental regulations. As Bujnovský (2015) stated, achieving the good ecological and chemical status of the land can have impact on improving water status and increasing the capacity of some ecological functions. The company manages and maintains forest land and is responsible for land improvements and stream dikes. In this case, the 'piggy-backing' principle is applied, where a single service generated by the water managing authority (MFE) is sold to one specific buyer (the local water management and supply company). The well-known example of PES for water from other European countries

Table 2. PFES characteristics – examples from the Slovak Republic.

	Land Tax Relief	**PWS, Military Forests**	**State Forestry Support**
Actors	Buyer: Municipality (Local level)	Buyer: Podtatranská waterworks services company	Buyer: State Agency (Agricultural Paying Agency)
	Seller: Forest owner/forest enterprise	Seller Military Forests and Estates of the SR, SOE	Seller: Forest owner/forest enterprise
	Intermediaries: -	Intermediaries: -	Intermediaries: Professional Licenced forest managers
	Knowledge Providers: -	Knowledge Providers: -	Knowledge Providers: Technical University in Zvolen, National Forest Center
ES provided	Biodiversity, scientific interest, recreation, water quality, soil protection	Water protection, Hydric-water management	Biodiversity, environmental quality, Supporting ES
Packaging of ES	Piggy-backing	Piggy-backing	Bundling
PES criteria	Other economic incentives:	PES-core schemes:	PES-like schemes
	Well-defined ES	Voluntary	Voluntary
	Minimum one buyer	Well-defined ES	Well defined land-use
	Minimum one provider	Minimum one buyer	Minimum one buyer
		Minimum one provider	Minimum one provider
		Conditionality on deliver ES	Conditionality on deliver ES

is implemented by Vittel (Nestlé Waters) in north-eastern France. This scheme illustrates how a 'narrow' PES would look like (Perrot-Maître, 2006).

PFES are also considered as tools to help maintain the multi-functional role of forests through supporting forest owners to adopt management practices that maximise environmental and social benefits (UNECE, 2014). Environmentally friendly or sustainable forest management has, nowadays, become a dominant theme in forestry throughout Europe. This paradigm recognises that forests are managed for a wide range of ecological, economic and social benefits (Cubbage et al., 2007). Various examples of uniform payments for environmentally friendly management practices could be found across European countries (OECD, 2010; Stanton et al., 2010; IUCN, 2009). These payments are direct financial support that affects the behaviour of forest owners through motivational stimuli (Šálka, 2006). The main aim of the Forestry Support for Non-Productive Forest Functions is to stimulate forest managers to ensure ecosystem services in the territory of the Slovak Republic in accordance with the forest management plan (Kicko, 2017). Financial support is, in principle, applicable to all FES (Šálka, Dobšinská, 2013). The state motivates forest owners or managers to apply sustainable forest management principles, which will lead to increased production of non-productive forest functions. Sustainable forest management (silviculture) creates multi-storeyed and rich mixed forest stands, with forest management emulating natural processes and seamless replacement of generations (Schütz et al., 2016). Understanding of the functions and processes of forest ecosystems is crucial for sustainable forest management practices (Machar, 2013). The government pays for the full package of ecosystem services that arise from the specific forestland according to the forest management plan. Smith et al. (2013) revealed that payments for sustainable forest management practices are made for a full suite of provided FES, as some proportions of the population will benefit from all of them. 'Bundling' of FES might provide a way for forest owners to get paid for more benefits they are providing (Deal et al., 2012). The concept of bundling can promote the integration of multiple ecological benefits (Collins, Larry, 2008; Venter et al., 2009; La Rocco, Deal, 2011).

Conclusion

Forests provide a wide range of ecosystem services. Unfortunately, many of the ecosystem services provided by forests are not directly paid for, and this may result in a lower provision than is socially optimal. The underlying reasons are that many ecosystem services are either public goods such as carbon sequestration or common goods such as water supply and, therefore, are non-market goods. Implementing PES schemes has become one of the policy strategies to ensure ecosystem services provision. Although several databases mapping PES schemes across Europe exist, they are either outdated or do not contain all relevant information.

Our analysis shows that only a few 'core' PES schemes are applied in Europe. Usually, the payments have a mixed public–private character. The European Union plays a strong role, and its Rural Development Fund provides financial support for biodiversity conservation or other environmental issues under the agro-environment pillar, which can be considered as a PES scheme.

Three types of public PFES schemes were identified in Slovakia: (1) forest land tax relief for protective and special purpose forests, (2) refunds for the restriction of ownership rights and (3) Forestry support for non-productive forest functions. Private PFES schemes have been identified so far only for watershed services and water quality improvement in MFE. As yet it is an open problem how appropriate payment schemes considering different types of forest ownerships and FES should look like and what kind of supporting policies are needed.

Acknowledgements

This article was supported by the Slovak Research and Development Agency (APVV-15-0715 and APVV-17-0232) and Internal Project Agency of Technical University in Zvolen (IPA 1/2018). The empirical results were obtained from the framework of COST CA15206 PESFOR-W.

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Ekológia (Bratislava) Vol. 38, No. 2, p. 166–177, 2019
DOI:10.2478/eko-2019-0013

FLORISTIC BIODIVERSITY OF WEED COMMUNITIES IN ARABLE LANDS OF ISTRIA PENINSULA (FROM 2005 TO 2017)

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Abstract

Štefanić E., Kovačević V., Antunović S., Japundžić-Palenkić B., Zima D., Turalija A., Nestorović N.: Floristic biodiversity of weed communities in arable lands of Istria peninsula (from 2005 to 2017). Ekológia (Bratislava), Vol. 38, No. 2, p. 166–177, 2019.

This paper analyses the floristic biodiversity of weed communities in the arable lands of the Istrian peninsula during a twelve year period (2005–2017). A total of 50 fields were surveyed for each sampling time using the seven-degree Braun-Blanquet cover abundance scale in the following agricultural categories: a) permanent crops (vineyards/olive groves), b) alfalfa fields, c) cereals, d) row crops and e) ruderal areas. The taxonomic identification was performed during the full development of vegetation, for cereals in June and July, and for the rest – in August and September. A total of 175 weed species were determined during both study periods with *Asteraceae* and *Poaceae* families as the most abundant. Altogether, therophytes were dominant in both surveys, followed by hemycryptophytes and geophytes. Variations in species composition were visible in both study periods (2005 and 2017) as well as in the selected habitat types. Exclusive species were found in addition to those that were common for both surveys. Changes in species composition between 2005 and 2017 referred to the difference in row spacing in earlier period, and ruderal vs. agricultural habitats in the recent survey. The differences in phenological traits between the past and present surveys were greatest for germination season in permanent crops and row crops, flowering start for permanent crops, flowering period for ruderal area and weed height for permanent crops. Significant differences between the past and present survey for other plant traits did not occur.

Key words: weeds, species shifts, relative change, troublesome index, weed functional traits.

Introduction

Arable weed vegetation represent a very dynamic and widespread system, but its biodiversity has been progressively changing in many agricultural areas worldwide (Rotches-

Ribalta et al., 2015, Chamorro et al., 2016, Burda, 2018). Since weeds pose a major threat to successful crop production, a lot of efforts have been carried out in intensification in agriculture during the last decades. Rapid changes in agricultural practices with intensive chemical fertilization and herbicide application, sowing high competitive crops, seed cleaning techniques and so on, had a significant impact on the number and abundance of arable weeds resulting in a huge decline in weed biodiversity (Sutclife, Kay, 2000; Barančok, Barančoková, 2016).

Surveying the flora of arable weeds has been a subject of many researches (Cirujeda et al., 2011., Kolarová et al., 2013) confirming that weeds are now among the most threatened groups of vascular plants (Storkey et al., 2012). A meta-analysis of changes in floristic composition of weed communities across Europe showed on average a 20% reduction of species per field between 1939 and 2012 (Richner et al., 2015). Several recent studies have been dealing with this problem and often led to contradictory results. For example, Lososová et al. (2004) explain that major changes in weed species composition in the Czech Republic and Slovakia were associated with a complex gradient of increasing altitude and precipitation and decreasing temperature and base status of the soil. Fried et al. (2008) suggested that major variations in species composition between fields were associated with crop type, while Pinke et al. (2010) explained that most of the variation inside the weed community could be explained by the seasonal aspect.

Change in diversity and composition of arable weed communities may also lead to changes in the community plant trait spectrum (Franke et al., 2009, Thompson et al., 1998). Plant traits, instead of species, are actually better adopted to particular specific cropping practices and agricultural habitats, hence, arranging weed species into functional groups may give a better understanding of how weed communities are assembled (Booth, Swanton, 2002).

This study aimed to compare the weed communities surveyed in 2005 and 2017 and to assess if the effect of weed shifting processes is visible in a twelve year period. The objective is to determine quantitative and qualitative relationships between crops and weed communities, and then to test the selected weed traits in the investigated agricultural categories to explore their functional response in a changing context.

Material and methods

Site description

Istria is the largest peninsula, situated in the north-west part of the Adriatic Sea, between the Gulf of Trieste and Kvarner Gulf. It spreads over three countries: Italy, Slovenia and Croatia, but the largest portion of the peninsula (89%) belongs to the Republic of Croatia, between latitude 45°15'24.00" N and longitude 13°54'9.59" E. The basic climate of the Istrian peninsula is Mediterranean. However, along the coast, it gradually changes towards the continent and it passes into continental climate, due to cold air circulating from the mountains and due to the vicinity of the Alps.

Istria has diverse landscapes and can be divided in three completely different areas. The hilly northern and north-eastern part of the peninsula – due to its scarce vegetation and nude Karst surfaces also known as White Istria. South-west from White Istria stretches considerably richer morphologically area. These are the lower flisch mountainous tracts consisting of impermeable marl, clay, and sandstone, which is why this part is called Grey Istria. Limestone terrace along the coastline, covered with red earth is called Red Istria. Agriculture has a long tradition in this territory, and nowadays a great attention is being given to the production of ecologic food, wine and olive growing.

Data sampling

A phytosociological relevés at a standard size of 100 m^2 surfaces were performed in each study period (2005 and 2017). Relevés were recorded in the centre of the fields to avoid the effect of neighbouring vegetation. A total of 50 fields were randomly selected in the so called 'gray' fertile inner land and 'red' coastal line part of the peninsula in 2005 and 2017, respectively. The fully developed vegetation was sampled as follows: for cereals in June and July, and for row crops, alfalfa, permanent crops and ruderal areas in August and September. All sampling sites shared the same aspect, soil bedrock, and a very similar altitude and slope. They were classified into the following agricultural categories: a) permanent crops (vineyards/olive groves), b) alfalfa fields, c) row crops, d) cereals and e) ruderal habitats.

The species coverage was estimated using the 7-degree Braun-Blanquet cover-abundance scale (Braun-Blanquet, 1964) and then transformed to an ordinal scale (Van Der Maarel, 1979), while their nomenclatural treatment mainly followed the check-list of Flora Croatica Database (https://hirc.botanic.hr/fcd/).

Data analysis

First, all the raw data were used for community evaluation, including the construction of the Venn diagram to determine the number of species distributed at or exclusive to each type of agricultural category and respective occurrence times. Then, to quantify the shift in weed species composition, a relative change of weed species was calculated using a method employed by Webster, Coble (1997) to compare the weeds surveys from 2005 and 2017 in permanent crops (vineyards/olive groves), alfalfa fields, row crops, cereals and ruderal habitats. Relative change (RC) for each species were calculated as follows: if a species that was the seventh most troublesome weed in past survey, and third most troublesome weed in recent survey, then the relative change would be +4. The weed that is not found in one of the surveys was considered to be the 11th most severe weed. The average change (AC) was calculated by dividing the relative change by the number of fields in the survey. Finally, the troublesome weed index (TWI) was calculated for both surveys (2005 and 2017) and consisted of the sum of rankings of weed species in each relevé.

This dataset was then used for multivariate analysis to identify factors (crop type) accounting for most of the variance within vegetation data. For that purpose, canonical discriminant analysis (CDA) was used to evaluate the association between agricultural categories on the occurrence of weed species using CANOCO 5 (ter Braak, Smilauer, 2012).

Finally, weed communities were analysed according to their functional traits. The eleven selected weed traits were obtained from the BiolFlor database (http://www.ufz.de/index.php) and included phenological traits (germination and flowering time and duration of the flowering season), traits relevant to plant competition (Storkey, 2006) like plant height together with Ellenberg indicator values for light, moisture and nutrient preferences (Ellenberg et al., 1992). Raunkiaer life forms was also added (Raunkiaer, 1934). For each agricultural category tested, mean comparisons of the past (2005) and present (2017) survey were made based on the mean selected traits using a two-sample t-test (H_0: vegetation in 2005 = vegetation in 2017).

Results

In total, 175 different weed species belonging to 36 families were found in the study area. In both surveys (2005 and 2017), the most representative families in terms of species richness were *Asteraceae*, *Poaceae* and *Fabceae* (Fig. 1). The domination of the *Asteraceae* family is particularly visible in 2017, *Fabaceae* shows a decrease in species richnes, while *Poaceae* remains stable without significant differences in their incidence throughout the study period.

Therophytes with 47% in 2005 and 51% in 2017 were a dominant life form, and the abundance of hemicryptophytes and geophytes were 37 and 11% in 2005 and 34 and 7% in 2017, respectively. Regarding the life forms in the selected agricultural categories, a slight increase of therophytes coupled to a reduction of hemicryptophytes occurred from 2005 to 2017 study periods in row crops and cereals (Fig. 2). However, permanent crops (vineyards and olive groves) increase the presence of geophytes in 2017, while the other life forms did not experience any significant variation.

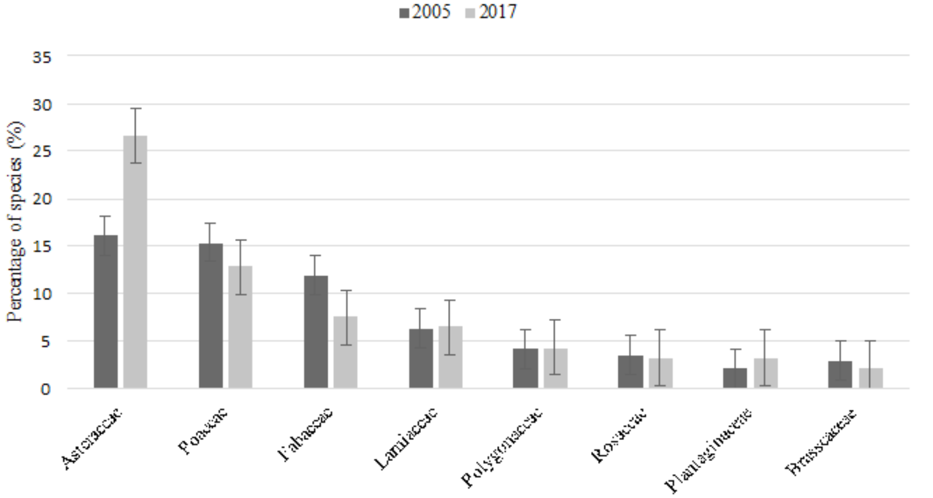

Fig. 1. Percentage of species in the main families of weeds found in the surveys during 2005 and 2017. Vertical bars represent the standard errors of means.

In both study periods (2005 and 2017), exclusive species were found in addition to those that were common to both surveys (Fig. 3). Altogether, there were 176 species found in this study. Richness was higher in 2005 (143 species), than in 2017 (97 species). Species common to both surveys were 64, but in earlier period, there were 79 species differentials that was not found in 2017. However, in the recent survey, 33 new weeds were discovered, which were not found in the 2005 appear.

Variation in weed species composition were also visible in the selected habitat types. For example: the richest ruderal weed community with a total of 99 species significantly decreased in richness from 85 found in 2005 to only 31 weed species in 2017 (Fig. 3). Only 17 species were common to both surveys. The weeds that have significantly declined in importance include *Ambrosia artemisiifolia* and *Lolium multiflorum* (Table 1). These two species were ranked as thirteenth and twelfth most troublesome weeds, respectively. Although *Avena fatua* was reduced 4 places in the relative rating, it was still ranked as the first troublesome weed (Table 1). Beside *A. fatua*, the up-and-coming troublesome weeds are also *Convolvulus arvensis* and *Chenopodium album*. These species have the biggest increases during the study period (RC: 11, and 9, respectively) and were ranked as the second and third most troublesome weeds in the ruderal areas (Table 1).

Permanent crops (vineyards and olive groves) were also floristically rich with a total of 86 species recorded throughout the study. The richest community was in 2017 having 66 species, compared to the past survey (2005) with 45 recorded weeds (Fig. 3). Common to both periods were 25 species, but 20 exclusive to the earlier period and 41 exclusive to the recent survey. The highest change happened to *Portulaca oleracea*, which decreases in its relative importance to the sixteenth place. In spite of this significant decrease in importance, it is still ranked on the sixth

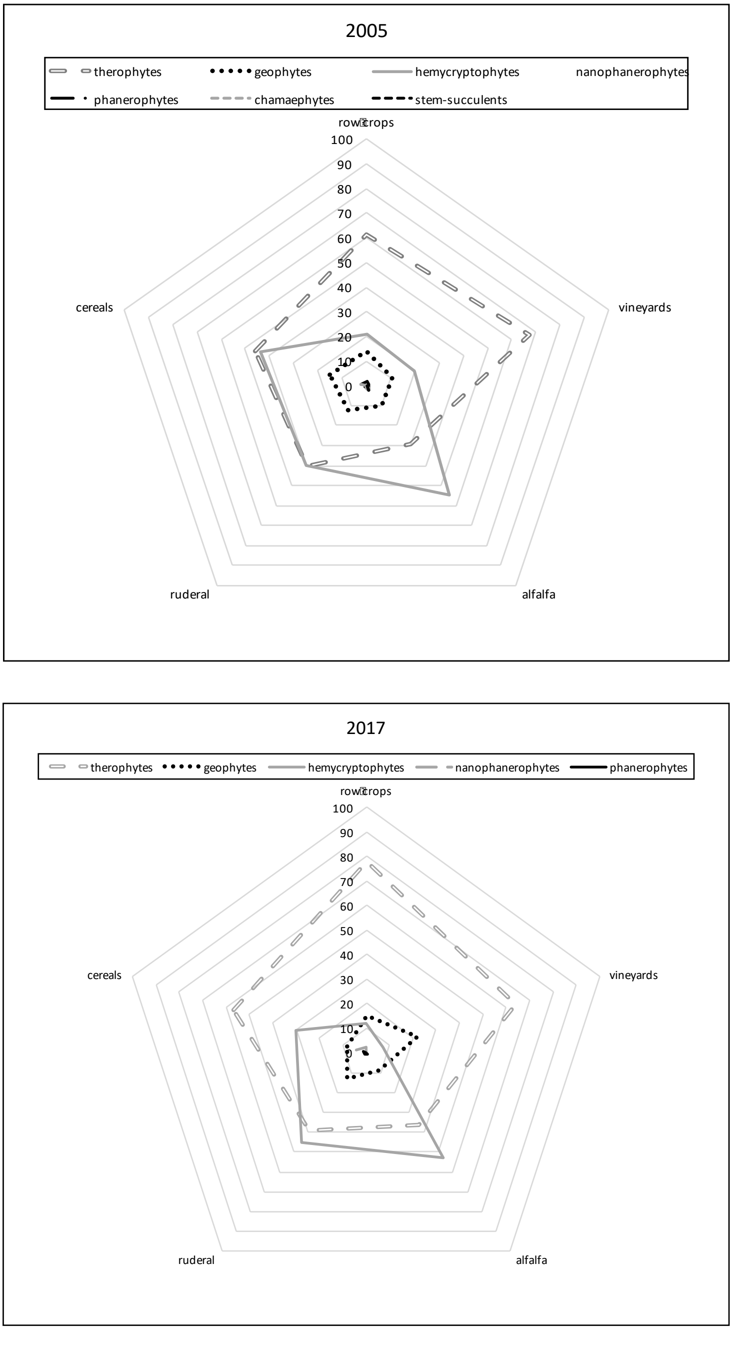

Fig. 2. Percentage of life forms in the investigated agricultural habitats in 2005 and 2017.

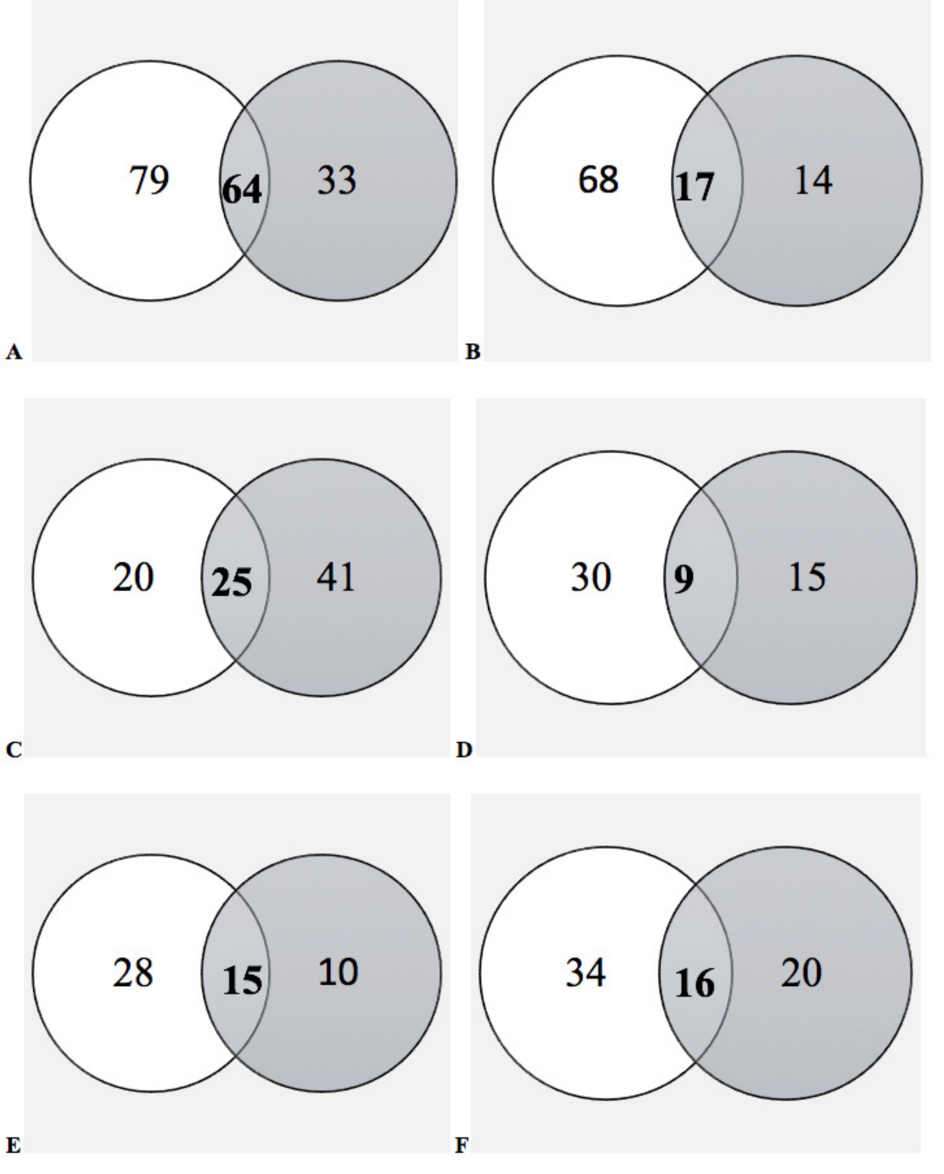

Fig. 3. Venn diagram with number of species of the weed communities found in 2005 (no fill) and 2017 (grey fill) in A: total area, B: ruderal area, C: permanent corps, D: alfalfa, E: cereals, F: row crops.

T a b l e 1. The relative rank of troublesome weeds in different agricultural habitats in Istria.

Scientific name of weed species	Agricultural habitat									
	Permanent crops		Alfalfa		Cereals		Row crops		Ruderal areas	
	RC (AC)*	TWI*	RC (AC)*	TWI*	RC (AC)*	TWI*	RC (AC)*	TWI*	RC (AC)*	TWI*
Amaranthus retroflexus	-8 (-1.1)	3	-	-	-	-	4 (1)	6	10 (10)	11
Ambrosia artemisiifolia	-	-	-	-	-	-	-2 (-2)	11	-10 (-10)	12
Avena fatua	-12 (-4)	9	-10 (-2)	4	-7 (-3.5)	5	-3 (-2.5)	13	-4 (-0.8)	1
Centaurea jacea	7 (1)	12	10 (10)	7	-	-	-	-	6 (6)	5
Chenopodium album	1 (0.2)	5	-	-	-	-	5 (1)	3	9 (3)	3
Convolvulus arvensis	8 (1)	1	2 (2.3)	6	2 (0.5)	1	1 (0.2)	1	11 (1.8)	2
Cynodon dactylon	13 (1.7)	2	7 (3.5)	1	-	-	-6 (-2)	4	5 (4.5)	6
Daucus carota	10 (1.4)	4	1 (0.5)	5	2 (0.5)	2	5 (2.5)	9	13(6.5)	9
Echinochloa crus-galli	-1 (-0.5)	11	-	-	-	-	6 (1.5)	5	-	-
Elymus repens	-	-	5 (5)	8	-	-	-	-	-2 (-1.9)	14
Lolium multiflorum	-	-	-4 (-2)	3	-	-	-	-	-6 (-2.7)	4
Panicum milliaceum	-	-	-	-	-	-	-8 (-7,5)	10	-	-
Polygonum aviculare	8 (2.7)	8	-	-	6 (3)	7	1 (0.3)	8	-4 (-2)	10
Polygonum persicaria	-5 (-5)	13	-	-	-10 (-10)	8	-1 (1)	7	-8 (-5.5)	13
Portulaca oleracea	-16 (-5.3)	6	-	-	-	-	-	-	13 (6.5)	7
Setria verticilata	-5 (-1.3)	10	8 (4)	2	-1 (-0.3)	3	7 (0.9)	2	11 (3.7)	8
Veronica persica	-10 (2.5)	7	-3 (-3)	9	-3 (-3)	6	-1 (-1)	12	1 (0.5)	15
Viola arvensis	-	-	-	-	-3 (-3)	4	-	-	-	-

Notes: Only species with mean relative abundance value ≥ 10.00 at least in one habitat per survey were presented.
*RC – relative change of species; AC – average change of species; TWI – troublesome weed index.

position as troublesome weed in permanent crops. The established troublesome weeds that have increased in importance were *Convolvulus arvensis* (RC: 8) and *Cynodon dactylon* (RC: 13) and were ranked on the first and second places as troublesome weeds in permanent crops (Table 1).

Alfalfa was the poorest weed community with a total of 54 determined species in both surveys. Weed community in 2005 comprised of 39 species, however, it significantly decreased in 2017 to 24 species (Fig. 3). There were only 9 species common to both surveys. The biggest increase in the weed ratings were found with *C. dactylon* and *Setaria verticilata* (Table 1).

Weed community in cereals comprised of a total of 53 species, having 43 weeds in the past, and 25 in the recent survey. Only 15 were common (Fig. 3). However, the most troublesome weeds *Convolvulus arvensis* and *Daucus carota* remain constant in their importance, and were ranked as the first and second most important weeds in cereals (Table 1).

A total of 70 weeds were recorded in row crops during both surveys (Figure 3). The number of species decrease from 50 in the past to 36 in the recent survey, and there were 16 species common to both study periods. The most troublesome weeds in row crops during the whole period is *Convolvulus arvensis,* while *Setaria verticilata* increased its importance (RC: 7) from the past survey and was ranked on the second place (Table 1). The highest relative change was observed for *Panicum milliaceum*, which decreased in importance and ranked on the 10th place.

Figures 4 and 5 show the variation in weed species composition in 2005 and 2017, as detected by CCA. Only species with the highest weight are displayed. Based on the ordination diagram in 2005 (Fig. 4), the first axis corresponded to row spacings and explained 27.9% of the total variation in species data, while the second axis mainly referred to the differences between ruderal vegetation and crops that explained additional 19.3% of the total variation.

In 2017, the first axis explained 32.1% of variation and clearly separated agricultural fields from

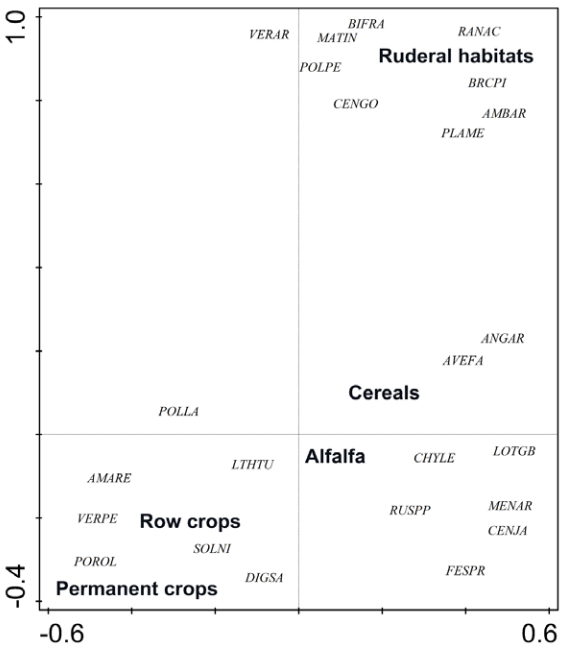

Fig. 4. Two-dimensional CCA ordination diagram of weed species in 2005 with explanatory variables. Species with low weight are not shown.

Notes: AMARE = *Amaranthus retroflexus,* AMBAR = *Ambrosia artemisiifolia*, ANGAR = *Anagalis arvensis*, AVEFA = *Avena fatua*, BRCPI = *Brachypodium pinnatum*, CENJA = *Centaurea jacea,* DIGSA = *Digitaria sanquinalis,* LOTGB = *Lotus glaber,* LTHLU = *Lathyrus tuberosus*, MENAR = *Mercurialis annua*, PLAME = Plantago media, POLLA = *Polygonum lapathifolium,* POROL = *Portulaca oleracea,* RANAC = *Ranunculus acris*, SOLNI = *Solanum nigrum*, VERAR = *Veronica arvensis*, VERPE = *Veronica persica*.

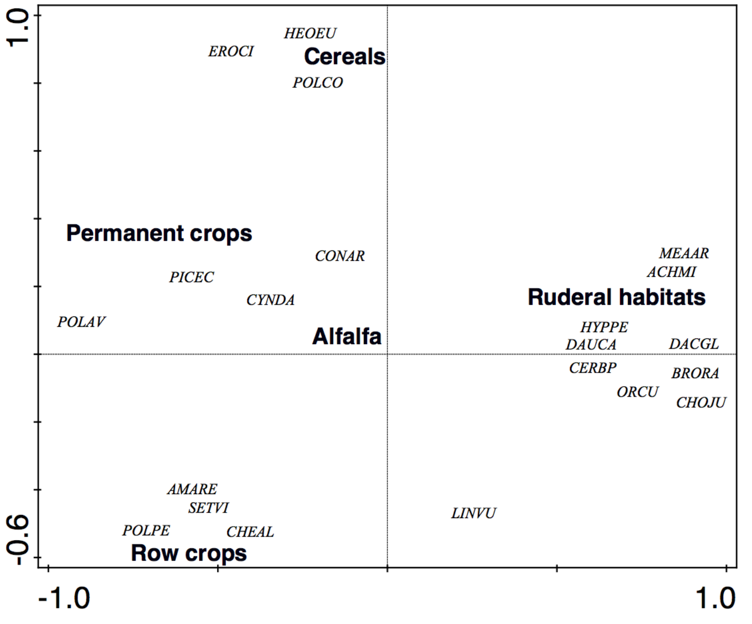

Fig. 5. Two-dimensional CCA ordination diagram of weed species in 2017 with explanatory variables. Species with low weight are not shown.
Notes: AMARE = *Amaranthus retroflexus*, ACHMI = *Achillea millefolium*, BRORA = *Bromus racemosus*, CARBP = *Capsella bursa-pastoris*, CHEAL = *Chenopodium album*, CHOJU = *Chondrila juncea*, CONAR = *Convolvulus arvensis*, CYNDA = *Cynodon dactylon*, DACGL = *Dactylis glomerata*, DAUCA = *Daucus carota*, EROCI = *Erodium cicutarium*, HEOEU = *Heliotropium europaeum*, HYPPE = *Hypericum perforatum*, ORCU = *Orobanche cumana*, PICEC = *Picris echioides*, POLAV = *Polygonum aviculare*, POLCO = *Falopia convolvulus*, POLPE = *Polygonum persicaria*, SETVI = *Setaria viridis*.

T a b l e 2. Differences in trait means of weed communities in arable lands of Istria peninsula between 2005 and 2017 sampling periods.

Trait means of weed communities	**Agricultural habitats**									
	Permanent crops		**Alfalfa**		**Cereals**		**Row crops**		**Ruderal habitats**	
	t-stat.	**P**	**t-stat.**	**P**	**t-stat.**	**P**	**t-stat.**	**P**	**t-stat.**	**P**
Germination season	-1.976	**0.044**	-0.211	0.335	-1.141	0.265	0.144	**0.045**	-0.626	0.536
Flowering start	-2.828	**0.007**	-0.485	0.633	-0.327	0.746	-0.201	0.842	-0.259	0.789
Flowering period	1.487	0.144	-0.319	0.753	-0.091	0.929	0.138	0.891	0.363	**0.041**
Height	-2.583	**0.013**	-0.195	0.847	0.845	0.407	0.987	0.330	1.236	0.226
Ellenberg moisture	1.593	0.118	-0.940	0.357	0.866	0.395	-1.227	0.228	1.422	0.165
Ellenberg light	0.496	0.632	0.439	0.665	0.253	0.802	-0.818	0.419	-0.528	0.601
Ellenberg N	1.914	0.062	-0.238	0.814	0.401	0.692	-0.818	0.419	-0.320	0.751

Notes: Bold type indicates statistical significance *P < 0.005 and **P < 0.001.

ruderal areas (Fig. 5). However, permanent crops and alfalfa were clustered together, whereas row crops and cereals were dispersed along axis 2, which explained 17.2% of total variation.

Table 2 shows the associations between investigated agricultural habitats (permanent crops, alfalfa, cereals, row crops and ruderal habitats) and the mean values of weed traits. The differences in paired comparisons between past and present surveys were greatest for germination season in permanent crops and row crops, flowering start for permanent crops, flowering period for ruderal area and weed height for permanent crops. Significant differences between the past and present survey for other plant traits did not occur.

Discussion

The most abundant families in this study, *Asteraceae* and *Poaceae,* were also reported not only as the most representative families in cultivated areas around the globe, but also as the families where many of the world's worst weeds belong and who are capable of producing large quantities of seeds with favourable dispersal mode and capability of colonization in various environments (Holm et al., 1977, 1991).

According to the life-cycle, most of the arable weed species are therophytes and survive as seeds during unfavourable seasons (Sutherland, 2004). After twelve years, the dominance of therophytes in this study became even more prominent, particularly in row crops and cereals, which is certainly the result of a strong anthropogenic impact, that is, an intensification of management practices in crop production (Cirujeda, 2011). Increasing of geophyte in permanent crops is probably related to their adaptation to minimum tillage, which is a widespread technique in this area nowadays (Karoglan Kontić et al., 1999). Moreover, a complex research performed in the Czech Republic and Slovakia indicated not only crops, but altitude, seasonal change and long-term change as the most important variables, which affected life forms and other vegetation characteristics (Lososova et al., 2004). For example, geophyte appears to be more frequent in root crops, therophytes were more common at lower altitudes and in earlier surveys, hemicryptophytes showed opposite patterns.

Weed vegetation, consisting predominantly of annual plants (therophytes), shows a much higher degree of temporal dynamics than other vegetation types (Lososova et al., 2004). Hence, shifts in weed population, and particularly decline of some species and increase in richness and abundance of others, were also detected during this investigation. Such a trend also occurred at field and regional scale in many European countries with intensive agricultural production (Baessler, Klotz, 2006; Richner et al., 2015). Consequences of the agricultural intensification are particularly visible in conventional agriculture leading to a much more rapid decline and loss in biodiversity than in organic agriculture (Flohre et al., 2011; Tilman et al., 2002).

The most troublesome weeds across all agricultural habitats in Istria is perennial vine *Convolvulus arvensis,* which has been shown to have relatively stable patches over time and crop rotations (Jurado-Exposito et al., 2004). An opposite trend was observed for *Avena fatua* which reduced in importance and particularly declined in permanent crops (-12 points) and alfalfa (-10 points). It probably happened due to the effectively combined diverse and optimal cultural practices and herbicide use against this annual weed (Harker et al., 2016).

Furthermore, comparing the pattern of the weed species composition between the selected agricultural habitats, a light discontinuity between the two sampling periods was observed. In the earlier survey (2005), the most significant influence on species composition occurred between the crop types. This indicates that different ecological conditions for weeds with regard to light conditions could affect floristic composition between wide row crops (permanent crops like orchards, olive grows and row crops) and narrow crops (alfalfa and cerelas) and ruderal habitats (Hallgren et al., 1999). Also, many other studies that cover a long-term period (Hallgren et al., 1999; Lososova et al., 2004) revealed that important changes have occurred in weed communities composition and were associated with a specific crop type. Human management factor was the main factor associated with the results from 2017. It showed that the main factor affecting the weed community composition was division between crops and ruderal area, and the second axis distinguished crop types. These results are in agreement with the study of Fried et al. (2008) indicating that a complex relationship in biotic and abiotic factors and their interactions exist between weed communities.

As a final step, the analyses of weed characteristic as community assembly based on the selection of functional traits revealed some changes between the 2005 and 2017 sampling period. In particular, a strong role of phenological characteristics in explaining the weed community shift was found. The main differences for average trait value between the past and recent survey is of earlier germination season and flowering period, and smaller weed heights for weed community in permanent crops in 2017.

In row crops, weed composition in 2017 consisted of weeds that germinated later in the season, and ruderal habitats consisted of weeds with longer flowering period. This phenological relationships is confirmed by the research done by Crowley (2004) that successful weed normally germinate around the time of the crop sown or flower before it is harvested, as well as timing of the tillage affects weed assemblage (Smith, 2006).

Acknowledgements

We are grateful to Ivana Borozni for correcting our writing style and grammar mistakes.

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Ekológia (Bratislava) Vol. 38, No. 2, p. 178–188, 2019
DOI:10.2478/eko-2019-0014

THE INFLUENCE OF TRANSPORT INFRASTRUCTURE DEVELOPMENT ON BIRD DIVERSITY AND ABUNDANCE

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Abstract

Rashidi M., Chamani A., Moshtaghi M.: The influence of transport infrastructure development on bird diversity and abundance. Ekológia (Bratislava), Vol. 38, No. 2, p. 178–188, 2019.

In recent decades, detrimental effects of roads have been the focus of numerous studies. Roadways have various negative effects, such as habitat fragmentation, noise and air pollution, on bird communities. This study was aimed to investigate the effects of traffic noise on the bird‘s abundance during autumn period. Field operations were performed in a forest located parallel to a main high-traffic highway. The bird‘s abundance was recorded using a point counting method at 27 points along three transects (65, 335 and 605 m from the road). The counting at each point was conducted every five minutes and repeated once every week (12 times during autumn). Environmental indices including the number of trees with DBH of over 20 cm, the number of standing dead trees, canopy cover percentage and Leq 30 were also measured. A total of 2950 bird belonging to 30 species were observed. The number of dominant species (more than 10) in the area considerably changed as the distance from the road increased. Leq 30 had the greatest correlation coefficient with bird abundance. Therefore, traffic noise has negative effects on the bird’s abundance in this area.

Key words: Leq 30, SPL, transect, traffic density.

Introduction

Diversity is the most important aspect of the community structure (Zhukov et al., 2018). The unceasing development of civilization and urbanization has changed the habitats of many animals especially birds (Wight, 2002). These changes exhibit pollution, soil destruction and plant formation, which leads to ecosystem transformation (Blinkova, Shupova, 2017). Degradation of migrating birds’ habitats has decreased numerous bird species (Šálek et al., 2010).

Natural and semi-natural forests as a buffer for urban ecosystems play a key role in reducing the negative impacts of anthropogenic impacts (Blinkova, Shupova, 2018).

In recent decades, the detrimental effects of anthropogenic noise on bird diversity have been the focus of numerous studies (Antze, Koper, 2018; Cardoso et al., 2018; Curry et al., 2018a; Kleist et al., 2018; Machado et al., 2017; Mulholland et al., 2018; Polak et al., 2013; Varasteh, 2011; Wiącek et al., 2015). Roadways have various negative effects on bird communities (Freed, Cann, 2009).

These effects include an increase in mortality rates (Mumme et al., 2000), changing the pattern of bird distribution due to constant traffic noise (Rao, Koli, 2017) and habitat fragmentation by creating a barrier to the birds' natural movement (Gerlach, Musolf, 2000; Summers et al., 2011).

Road networks can separate animal population into subpopulations which severely constrain their genetic flow due to inbreeding (Peris, Pescador, 2004). Based on habitat type and road characteristics (Forman et al., 2002), these negative effects, may reach up to 10 or 100 m away from a particular habitat. Birds generally avoid habitats manipulated by human, as it has negative effects on their nutrition, survival, and abundance (Fernández-Juricic, 2002).

The annual cycle of bird migration, lasts for nearly one-third of the year (Sauer et al., 2011). During this period, birds spend considerably more time at stopover sites (Wikelski et al., 2003). Food seeking and resting may cover 95% of their migration time (Alerstam, 2003). Human activities are one of the most important factors affecting the migration of birds and the time they spend at stopover sites. The effects of noise pollution on species depend on age, gender, species history, habitat type, season, activity time and so on (Summers et al., 2011). The long-term implications of chronic noise exposure, on adult and nestling corticosterone levels, and nestling body condition has been proved (Injaian et al., 2018). Some birds in proximity to sound, have mechanisms for modulating their effects. But the usefulness of these mechanisms varies, based on physiological differences between different species (Curry et al., 2018b).

Such issues have raised major concerns about the sustainability and survival of wildlife populations near the roads (Polak et al., 2013). Therefore, this study was aimed to investigate the effects of traffic noise on the diversity and abundance of bird populations in urban ecosystems.

Materials and methods

Study area

This study, was performed in Isfahan, a city located 30–50 km from the centre of Isfahan Province covering an area of 34,500 ha (32°30'20" N, 51°30'15" E-32°48'10" N, 51°47'10" E). The studied forest is located parallel to a main high-traffic highway. The dominant tree species in this area are *Pinus* sp., *Salix* sp., *Acer* sp., *Melia azedarach*, *Platanus racemose*, *Myrtus communis*, *Tamarix ramosissima* and *Elaeagnus* sp. During the study period, the average traffic intensity was 1106 personal vehicles, 53 heavy vehicles, and 92 motorcycles per hour. The speed limit in this highway is 60 km/h.

The number and distances of transects

Three transects (respectively 65, 335, and 605 m from the road) were selected considering the forest area. The transverse and longitudinal distances of the transects were 270 and 150 m, respectively. Nine points in each transect, with 75 m distances, were determined along each transect (A total of 27 points). The bird's abundance along each transect were estimated every 5 minutes using the point count method. Based on the transverse and longitudinal distances of the transects also the tree cover density and visibility, all birds were recorded within a 50 m radius (West et al., 2002; Wiącek et al., 2015).

Numbers of birds

The birds were observed and identified using a binocular. The observations began in autumn 2016, under favourable weather conditions. That is, in the absence of rainfall or extreme wind blows. The counting started at sunrise, when birds had maximum activity and were most likely to be seen, and continued until 2 p.m. (Mammides et al., 2017). In order to estimate the species' abundance, the numbers of species identified in the three transects were counted and recorded. Counting at each point was done once every week (12 times during autumn). All observations were made by two expert observers. All the birds were identified with the help of the Iranian bird guides. To eliminate possible errors in the observation hours, observations were conducted in a different order as described by (Wiącek et al., 2015). Each day, the observation began from the point it had ended the day before. In addition, since birds with different diets have different ecological niches, the birds were categorized into three: granivorous, insectivores, and omnivores (Polak et al., 2013).

Environmental parameters

The environmental indices, including the number of trees with a diameter at breast height (DBH) of over 20 cm, number of dead trees (NDT), canopy cover percentage and temperature, were measured in all transects. In order to calculate the trees' DBH, the diameter of each tree was measured at breast height (1.3 m) and the DBH was obtained using the circle circumference formula. In order to determine the canopy cover percentage, a measuring tape was used to measure the radius of each tree's shadow in favourable, sunny conditions when the tree shadows could be distinguished. The area of the shadows was then calculated and divided by the area of each transect. Finally, the temperature data were collected from the weather station in the area.

Sound measurement

The equivalent sound level (Leq30) represents the estimated sound average over a period of 30 min. Leq30 was used in this study because least error was detected during the measurement of equivalent sound level (Wang et al., 2005). Leq30 was measured at the central station of each transect for over a 5-15-min period using a sound level meter model TES 1353. To minimize the effects of ground vibration factors, the measurements were performed a the height of 1-2 m above the ground (Machado et al., 2017). The sound pressure level (SPL), minimum, maximum, and average sound level (as the most important factor used), and Leq30 were also estimated during this interval. Vehicle traffic rates were also camera recorded and calculated throughout the sound measurement period. To determine day(s) with higher noise pollution (peak days), the traffic rates were measured daily during the week. The traffic rate was measured by direct observation and recorded with hand camera. Traffic vehicles were then divided into three different classes including trucks, cars, and motorcycles.

Statistical analysis

The homogeneity and normal distribution of data were analysed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). DISTANCE 6.0 was used to calculate the bird density. The relationship between the bird abundance and environmental variables were investigated using CANOCO. Other statistical analyses (ANOVA, ANCOVA, PCA, correlation coefficients and so on), were conducted using STATISTICA 10.0.

Results and discussion

A total of 2950 bird belonging to 30 species were observed in the study area (Table 1). The most dominant order was *Passeriformes*. The least abundance belonged to *Piciformes, Coraciiformes*, and *Psittaciformes.* All the observed species was in the Least Concern category (IUCN). The environmental indices in the area are shown in Table 2. According to the Shapiro-Wilk test, data from the three transects had normal distribution ($P = 0.205$, 0.174, and 0.579 for transects 1–3, respectively) and were analysed using the parametric tests. Moreover, Levene's test confirmed the homogeneity of the data ($F_1 = 2$, $F_2 = 15$; $P = 0.132$). One-way ANOVA and Duncan's test ($F = 36.24$; $P < 0.05$) showed a significant difference in the mean species presence among the three transects.

Figure 1 shows significantly increasing species richness with distances from the road. The number of dominant species (>10) in the region considerably changed with distance from the road. The abundance of *Columba palumbus, Psittacula krameri*, *Corvus frugilegus* and *C. corone* increased with distance from the road. While some species, such as *Motacilla alba, Spilopelia senegalensis* and *Motacilla flava,* preferred the middle transect.

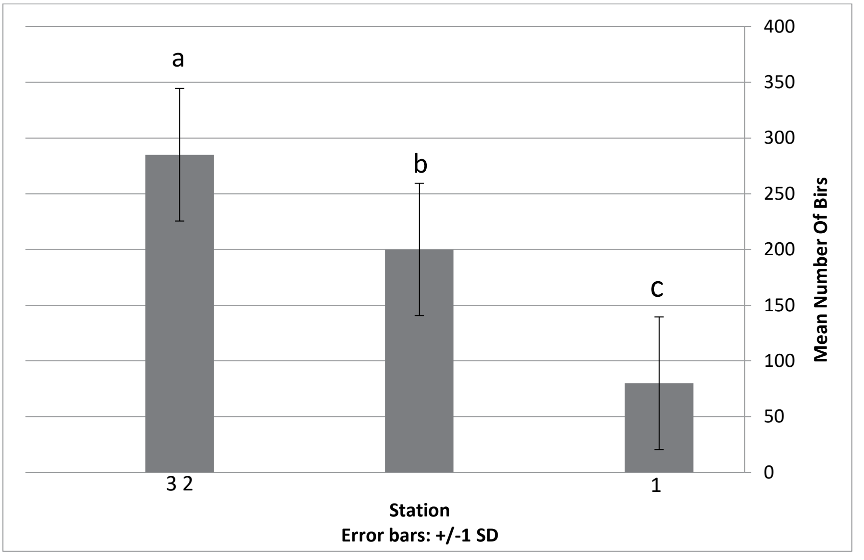

Fig. 1. The mean and standard deviation of bird abundance in transects 1–3 (different letters, indicate significant differences at the 0.05 level).

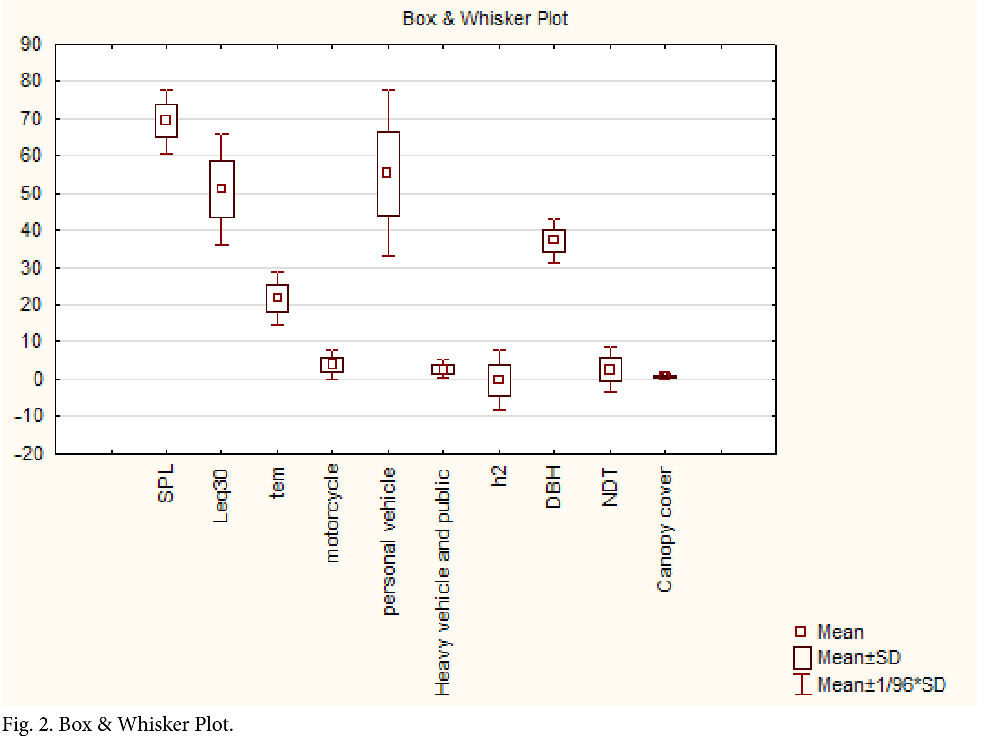

Fig. 2. Box & Whisker Plot.

T a b l e 1. The observed and identified species in study area.

Species	Total number	Foraging guild	Number of individuals		
			T1	T2	T3
Corvus corone	400	O	75	107	218
Pica pica	192	O	59	66	67
Motacilla alba	570	G	132	249	189
Corvus florensis	791	O	38	144	609
Passer domesticus	648	G	105	292	254
Phylloscopus collybita	47	I	3	21	48
Accipiter nisus	1	-	1	0	0
Tringa solitaria	314	-	0	277	37
Actitis hypoleucos	2	I	0	1	1
Tringa totanus	2	-	0	1	1
Spilopelia senegalensis	21	G	2	12	7
Gallinago gallinago	21	I	0	12	9
Pyrrhocorax pyrrhocorax	1	O	0	0	1
Motacilla cinerea	3	G	0	0	3
Acrocephalus arundinaceus	4	I	0	0	4
Sylvia atricapilla	4	I	0	0	4
Dendrocopos syriacus	11	G	5	3	3
Accipiter brevipes	2	-	0	0	2
Egretta garzetta	12	I	0	0	12
Hippolais caligata	14	I	8	2	4
Circus pygargus	1	I	0	0	1
Galerida cristata	27	G	2	10	15
Gallinula chloropus	45	G	0	22	23
Hippolais languida	10	I	0	7	3
Upupa epops	1	I	0	1	0
Psittacula krameri	14	G	0	2	12
Columba livia	12	G	6	6	0
Columba palumbus	109	G	8	5	96
Motacilla citreola	3	G	0	1	2
Motacilla flava feldegg	10	G	0	10	0
Acridotheres tristis	9	O	0	0	9
Falco tinnunculus	1	-	0	0	1
Fringilla coelebs	2	G	0	2	0
Anthus campestris	1	I	0	1	0
Anas platyrhynchos	5	O	0	5	0
Luscinia svecica	1	I	0	0	1
Larus ridibundus	17	G	0	0	17
Lanius collurio	1	I	0	0	1
Total	**3329**		**444**	**1249**	**1618**

T a b l e 2. Environmental indices in the area.

Variable	T1	T2	T3
SPL	*75.5*	*68.1*	*65.1*
Leq30	*60.7*	*46.8*	*45.3*
Motorcycle	*23*	*0*	*0*
Personal vehicle	*55.3*	*0*	*0*
Heavy vehicle and public	*2.6*	*0*	*0*
DBH	25/62	33.22	7.8
NDT	11	2	2
Canopy cover	0/78	0/44	1/18
H	1587.333	1582.333	1582
Δh	**-5**	**-0.333**	**5.333333**

Figure 2 shows the mean and standard deviation of the environmental variables. Accordingly, the sound parameters are in the same range with the vehicle traffic and environmental parameters.

As shown in Figs 3 and 4, SPL and Leq30 was significantly decreased with distance from the road. While Leq30 in transect 1 had significant differences with transects 2 and 3, no other significant differences in Leq30 were observed.

As shown in Figure 5, four Principal Components (PCs) explained 65.4% of the total variation. Of this total, 18.9% was explained by PC1 in which Leq30, NDT, DBH, distance from the road and heavy vehicle were mainly responsible for this variation; 18.4% can be explained by PC2 which is mainly attributed to SPL and personal vehicle; 10.6% was explained by PC3 can mainly be attributed to temperature, motorcycle and h; and 5% was explained by PC4 in which canopy cover was more important.

Since regression models (Table 3) are used to prioritize the effect size of variables, they can be used to clarify the most important factors involved in the presence of specific species by calculating the differences between the R values. Moreover, the mean square (MS) can determine the effects of factors on each other.

According to the results, Leq30 had the highest correlation coefficient with species richness. Therefore, traffic noise had negative effects on the bird abundance and diversity in this area. Specifically, motorcycles were found to produce evasive sounds beyond the normal levels and ensure the absence of bird species.

Discussion

Biodiversity indices facilitate the identification of factors affecting habitats and serve as a tool for monitoring and evaluation of changes in ecosystem (Ramp et al., 2005). Based on the PCA results, DBH, NDT, Leq30, and distance from the road had the greatest impacts on the abundance and diversity of bird species. Similarly, (McClure et al., 2013) found decreased abundance and diversity of species in areas adjacent to roads. In the present study, the lowest abundance was observed in the first transect located nearest to the road with maximum levels of traffic noise. The presence of

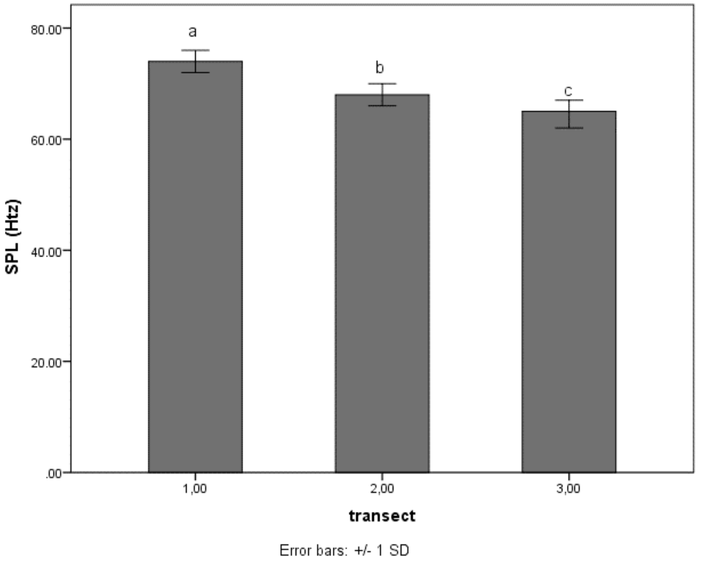

Fig. 3. The mean and standard deviation of SPL in transects 1–3 (different letters, indicate significant differences at the 0.05 level).

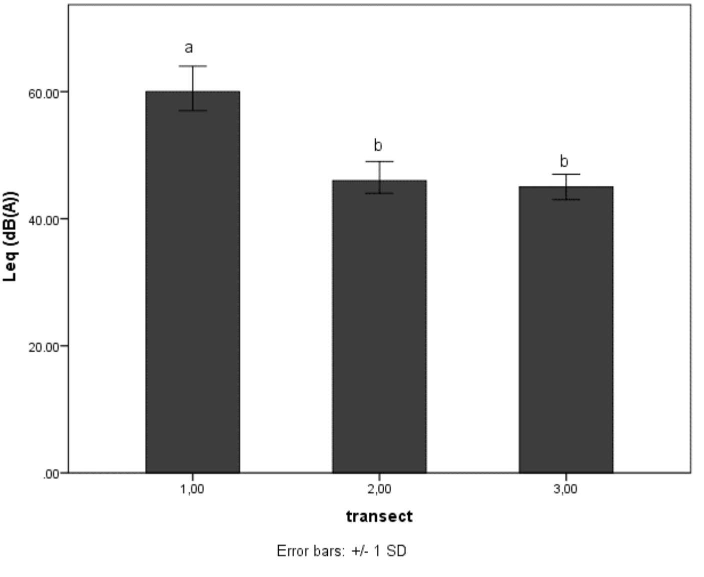

Fig. 4. The mean and standard deviation of Leq $_{30}$ in transects 1–3 (different letters, indicate significant differences at the 0.05 level).

most species increased with the distance from the road as a result of greater security and silence in the second and third transects. Thus, as reported by Polak et al. (2013), roads act as a limiting factor for the presence of species.

Based on the results, motor vehicle traffic rates had direct correlations with Leq30 and SPL. Personal vehicles had the greatest impacts and motor vehicles had the least significant effects on SPL. In other words, higher traffic was associated with greater levels of sound equivalent and pressure. Moreover, Leq30 had the highest correlation with the diversity of species. Therefore, the noise pollution caused by traffic negatively affected the bird species' diversity and abundance in the region.

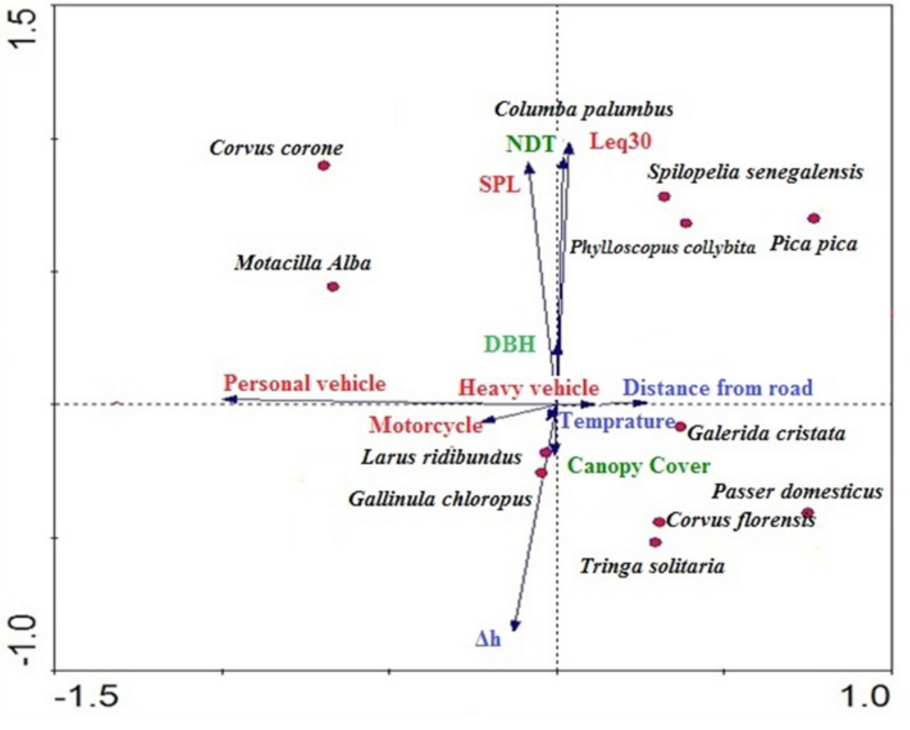

Fig. 5. Ordination diagram of PCA with 12 of the highest bird species richness.

Among the vegetation factors, NDT was the most effective parameter (correlation=1) in increasing the abundance of species, such as *Pica pica* and *Corvus corone*, which nested on dead trees. Noise pollution and vehicle traffic rates did not play significant roles in the presence of these species. This highlighted the importance of NDT and the need for the preservation of dead trees in the habitats of the mentioned species (Sillett, Holmes, 2002). Sillett and Holmes (2002), in their study, identified the preservation of at least six dead trees per hectare (one with a diameter ≥ 50 cm and others with a diameter ≥ 40 cm) as a valuable management strategy.

T a b l e 3. Modelling the regression correlation coefficients between sound-dependent environmental factors.

Dependent Variable	Multiple R	Multiple R2	Letter Adjusted R2	SS Model	df Model	SS Residual	Df Residual	MS Residual	MS Residual	F	p
SPL	0.44	0.2	0.15	0.68	1	68.05	269.55	16	16.84	4.03	0.06
Leq 30	0.61	0.3	0.34	375.38	1	375.38	613.21	16	38.32	9.79	0.006
temp	0.076	0.005	-0.05	1.3	1	1.33	223.83	16	13.98	0.09	0.76
motorcycle	0.51	0.26	0.22	20.05	1	20.05	55.44	16	3.4	5.89	0.02
Personal vehicle	0.43	0.18	0.13	410.88	1	410.88	178.31	16	111.44	3.68	0.07
Heavy vehicle and public	0.35	0.12	0.07	3.55	1	3.55	24.44	16	1.52	2.32	0.14

Shelter is a major biological need for most birds. This need was clearly detected in the case of *Psittacula krameri,* whose presence was mostly observed in the third transect near the willow community. The presence of *Salix* sp. in the second and third transects can be a very good shelter for birds to create different ecological niches. The presence of *Motacilla flava* was observed in the second transect where *Tamarix ramosissima* existed. The presence of *Acridotheres tristis* was observed in the third transect owing to the existence of *Populus euphratica* and *Platanus* sp. Higher percentage of vegetation canopy increased the abundance of species, such as *Phylloscopus collybita* and *Dendrocopos syriacus* for which canopy is a key factor in habitat selection. Shade-friendly bird species, such as *Motacilla alba*, would be forced to change their distribution range to areas near roads where canopy cover is higher than the average level (0.78 vs. 14.4%) due to the presence of planted species. This can provide a safe shelter for vulnerable species, such as *M. alba*. Hence, some species prioritize shelter over other habitat parameters. Furthermore, canopy cover is more effective than traffic rate and noise pollution in their habitat selection. According to other studies, depending on the species, roads can either decrease (Forman et al., 2002) or increase the diversity of birds (Meunier et al., 1999).

Furthermore, changes in elevation in an ecosystem can also justify altered birds' fauna in the studied transects. Since the average temperature was constant during the study period, it did not directly affect the presence or absence of species. DBH and tree height are also other important variables in the habitat structure and can be individually used in other investigations due to homogeneity of variances. (Díaz, 2006) emphasized the positive relationships between habitat parameters and species diversity and abundance (Morelli et al., 2014) concluded that changes in the habitat flora was associated with changes in birds' fauna and that the presence of species was limited by the specific habitat conditions in each area. Increasing the area, safety and diversity of aquatic plants play important roles in attracting more aquatic and offshore birds (Fox, Bell, 1994). Cavity-nesting birds, which are dependent upon dead trees with specific diameter, height, and degrees of corruption, are the most vulnerable to the marginal effects of roads. Elimination of these parameters will increase the vulnerability of primary cavity-nesting birds, such as woodpeckers (Kilgo, 2005).

The results of this study can effectively help in the planning, management, and development of road construction in forest areas where the protection of bird species from noise pollution is intended.

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Ekológia (Bratislava) Vol. 38, No. 2, p. 189–200, 2019
DOI:10.2478/eko-2019-0015

THE CHARACTERISTICS OF AFTERSHOCK ACTIVITIES OF DIEN BIEN EARTHQUAKE ON 19 FEBRUARY 2001 AND THEIR RELATION TO THE LOCAL GEOMORPHOLOGICAL, TECTONIC FEATURES

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Abstract

Ngo Thi Lu, Phung Thi Thu Hang, Nguyen Huu Tuyen, Ha Thi Giang, Nguyen Thanh Hai: The characteristics of aftershock activities of Dien Bien earthquake on 19 February 2001 and their relation to the local geomorphological, tectonic features. Ekológia (Bratislava), Vol. 38, No. 2, p. 189–200, 2019.

This article examines in detail the characteristics of Dien Bien earthquake on 19 February 2001 and its aftershocks. On the basis of the temporal development of aftershocks and the spatial distribution of tectonic faults, five aftershock series have been determined. The analysis of spatial distribution and temporal evolution of these five aftershock series has clarified the development in the source zone of Dien Bien earthquake, which is closely related to the active and recent activities of tectonic faults in the area, especially Lai Chau Dien Bien fault. The comparison between characteristics of aftershock activities of Dien Bien earthquake and geomorphological features as well as tectonic activities in the area has indicated that the magnitude of these aftershocks and their temporal evolution (early or late) depend not only on the closer or further distance compared to the mainshock and the active faults that cause them but also on terrain elevation, slope index, lineament density and their positions relative to other tectonic faults in the studied area.

Key words: aftershocks, Dien Bien earthquake, morphotectonic.

Introduction

On 19 February 2001, at 22:51 (GMT), an earthquake with magnitude M = 5.3 occurred 20 km southwest of Dien Bien Phu. According to the data from International Seismological Centre (ISC), this earthquake was recorded by many international seismic stations in the area. Dien Bien earthquake was accompanied by many aftershocks, some of which had the magnitude of $4 \leq M \leq 4.8$. It caused much damage to Dien Bien Phu and adjacent areas.

Since the occurrence of this earthquake, a number of studies have been conducted in different directions such as investigating the impact of the earthquake, measuring the microseismic oscillations, assessing the ground impact as well as establishing the isoseismic map and examining the aftershock activities (Le Tu Son et al., 2004; Cao Dinh Trieu, 2001; Nguyen Ngoc Thuy et al., 2005). However, these studies are mainly based on seismic data and have not elucidated the development in the hypocentre region as well as its relation to the geomorphological features and the tectonic activities in the area.

Recently, there have been many studies evaluating seismic hazard and partitioning of strong earthquake activity (Mmax) based on the combination of geological, geophysical, geomorphological and seismic data (Ngo Thi Lu et al., 2016, 2017; Nguyen Huu Tuyen, Ngo Thi Lu, 2012; Rodkin et al., 2014).

In addition, the development of science and information technology allows us to use geographic information system,GIS and remote sensing data in a series of studies, such as the risk assessment of landslide risk (Barančoková, Kenderessy, 2014); to use GIS for creation of geohazards map (Magulová, 2009); using modern geological techniques and spatial analysis such as satellite imagery and geographic information to study, assess and establish maps of earthquake risk (Nguyen Hong Phuong et al., 2018), maps of the forest fires risk (Belgherbi et al., 2018); and so on.

On the other hand, with a large number of accompanying aftershocks as mentioned in the aftershock catalogue of Dien Bien earthquake (Le Tu Son et al., 2004), the more detailed studies on the characteristics of aftershock activities and their development in the hypocentre region can be conducted. It allows clarifying the recent activities of a series of tectonic faults in the area. Therefore, in this article, we have investigated the characteristics of aftershock activities of Dien Bien earthquake on 19 February 2001 and studied their relation to geomorphological features and tectonic activities based on the modern geological techniques and combination of seismic data and remote sensing data.

Methods

Seismic data

To study the characteristics of aftershock activities of Dien Bien earthquake, first, we have collected the data on its aftershocks. According to data in the aftershock catalogue of Dien Bien earthquake (Le Tu Son et al., 2004), from 19 February 2001 to 9 June 2001, 378 aftershocks with a magnitude of $M \leq 4.8$ have occurred. The earthquake magnitude (M) is determined by the length of recorded tape in accordance with (Nguyen Dinh Xuyen, Nguyen Le Yem, 1980):

$$M_T = 2.67 \lg T - 2.49 \quad (1)$$

where T is the length of recorded tape (in second).

On the basis of the experience of seismic data processing staff at the Institute of Geophysics, Vietnam, the length of recorded tape is calculated from the occurrence of wave to the end on the tape (when the wave amplitude is twice as high as the interference amplitude). In addition, this earthquake was also recorded by many international seismic stations in the area (Seismological Bulletin of ISC, 2001).

Amongst 378 aftershocks mentioned above, 29 aftershocks had a magnitude of $M \geq 2.0$, in which 23 aftershocks had a magnitude of $M \geq 2.5$. We have studied the temporal evolution of these aftershocks as well as their spatial distribution. We have also examined their characteristics in the general relation to other strong earthquakes and aftershocks in the adjacent areas (Le Tu Son et al., 2004). The data on Dien Bien earthquake and its aftershocks are presented in Table 1. It should be noted that in Table 1, the magnitude values M_T according to the length of recorded tape have been converted to the magnitude values M_S according to the surface wave using the following formula (Ngo Thi Lu, 2003):

$$M_S = 0.98\ M_T + 0.10$$

Furthermore, we have added the dimension parameters of hypocentre of mainshock, which are calculated using the following formula (Shebalin, 1971, 1974) in accordance with (Ngo Thi Lu, 1999):

$$\lg L = 0.55\ M - 2.0$$

$$\lg W = 0.30\ M - 0.75$$

where L is the length of hypocentre (km) and W is the width of hypocentre (km).

T a b l e 1. Basic parameters of Dien Bien earthquake and its aftershocks.

No.	**Data**	**h. m. s.**	**ϕ °N**	**λ°E**	**h (km)**	***MS***	**L (km)**	**W (km)**
*(mainshock)	19 02 01	15 51 35.2	21.331	102.897	6.1	5.3	6.92	8.22
1	19 02 01	16 07 02.3	21.339	102.934	4.3	3.1		
2	19 02 01	16 15 50.3	21.345	102.941	0	2.6		
3	19 02 01	16 40 18.9	21.298	102.927	0.8	4.2		
4	19 02 01	16 41 18.4	21.325	102.91	0	4.2		
5	19 02 01	19 02 50.1	21.305	102.903	0	4.8		
6	19 02 01	19 02 51.7	21.377	102.997	10	2.3		
7	19 02 01	22 58 31.5	21.497	102.886	1.2	2.8		
8	21 02 01	11 03 45.0	21.395	102.991	0	3.2		
9	22 02 01	11 36 33.5	21.352	102.925	1.8	3.1		
10	23 02 01	17 53 28.9	21.518	102.916	0	3.1		
11	24 02 01	22 14 31.1	21.335	102.928	0	3.6		
12	24 02 01	22 38 42.5	21.587	102.915	6.5	2.9		
13	04 03 01	20 18 46.3	21.539	102.652	10	2.7		
14	04 03 01	20 19 49.0	21.315	102.822	0	4.0		
15	04 03 01	20 40 55.7	21.352	102.981	10	2.2		
16	04 03 01	20 41 53.7	21.447	102.88	0	2.9		
17	05 03 01	02 12 07.8	21.343	102.99	10	2.6		
18	05 03 01	02 13 03.7	21.48	102.795	0	2.7		
19	05 03 01	14 22 37.9	21.401	102.986	10	3.1		
20	05 03 01	14 23 39.2	21.4	103.102	0.3	2.8		
21	05 03 01	15 07 59.6	21.39	102.89	5.3	3.2		
22	25 03 01	13 28 22.2	21.3	103.011	0	2.9		
23	26 03 01	22 49 50.4	21.47	103.093	10	2.2		
24	03 04 01	04 30 06.9	21.415	103.16	10	2.3		
25	22 04 01	12 30 50.2	21.292	102.909	0	3.3		
26	23 04 01	15 51 16.8	21.31	102.825	0	3.8		
27	02 05 01	14 03 14.5	21.318	103.09	0	2.3		
28	08 06 01	07 38 13.6	21.338	102.946	1.8	3		
29	08 06 01	14 09 22.6	21.233	103.058	0	2.1		

Morphotectonic data

To study the relationship between the aftershocks of Dien Bien earthquake and tectonic activity in the area, we have analysed morphotectonic features of the restricted area that is limited by the coordinates: φ = 21.06–21.91°N; λ = 102.70–103.39°E (Figures 3 and 4).

Large-scale topographic maps (1/50,000), digital elevation maps and remote sensing data of the Digital Elevation Model (DEM images) were used to analyse the density of lineaments and geomorphic indicators as the first step to detect the surface deformation associated with tectonic activity.

The V_f index (*the ratio between the valley floor width and its height)* reflects deepening of erosion. It allows to estimate the rate of vertical movement of the area. The lower values of V_f (often close to values 0 with V-shaped valleys) are commonly related to an active uplifted area (Bull, 2007; Bull, McFadden, 1977).

The analysing of remote sensing data includes aerial photography and satellite images with high resolution in order to reveal landscape deformation that are formed by inter-activities of the endogenous–exogenous processes. The morphotectonic indicators for horizontal movement are rectilinear fault scarps, beheaded valleys of minor tributaries, as well as shutter ridges. And component of normal slip is identified by the presence of small-size triangular facets (Zuchiewicz, Cuong, 2002, 2003). Moreover, tectonic data are referenced from the tectonic map in (Nguyen Ngoc Thuy et al., 2005).

Characteristics of aftershock activities of Dien Bien earthquake

Spatial distribution of aftershocks

Fig. 1 shows the distribution of epicentres of Dien Bien earthquake and its aftershocks. The aftershocks are numbered from 1 to 29 according to the temporal evolution in the hypocentre (their occurrence time). In consideration of their relation to tectonic faults in the area, it is obvious from Fig. 1 that the major earthquake occurred at the coordinate φ = 21.336, λ = 102.803, near Lai Chau-Dien Bien (LC-DB) fault, slightly towards the right of this fault. According to the results obtained in Figure 1, the epicentres of aftershocks of Dien Bien earthquake were distributed along this fault, mainly in the northeast (NE)–southwest (SW) direction in an area of about 30 km wide and more than 40 km long from Thanh Nưa (NE) to Tay Trang (SW), and concentrated more densely in Dien Bien Phu. In addition, the epicentres of aftershocks with greater magnitude were concentrated in a region on the right of this fault with the narrower area of more than 50 km^2, more than 10 km long and about 5 km wide. The aftershocks with smaller magnitude were distributed outwards on both sides of LC-DB fault and more northwards compared to the mainshock. It is also observed that the LC-DB fault crosses this area in the NE–SW direction and its branch runs in the sub-longitudinal direction. The epicentre of Dien Bien earthquake was very close to the intersection of LC-DB fault with other faults such as Dien Bien-Pakanua, Fumaytun, Thanh Nua-Keo Lom, Kan Ho-Muong Mon faults (Fig.1).

Temporal evolution of aftershocks

In consideration of temporal evolution of aftershocks and based on the distribution of tectonic faults in the area, it is possible to delineate five epicentre regions of aftershocks of Dien Bien earthquake as in Fig. 1 (below called aftershock series).

- ***The first aftershock series (I):*** This series includes the earliest aftershocks following the mainshock. Their epicentres were distributed quite concentratedly in an ellipse-shaped area

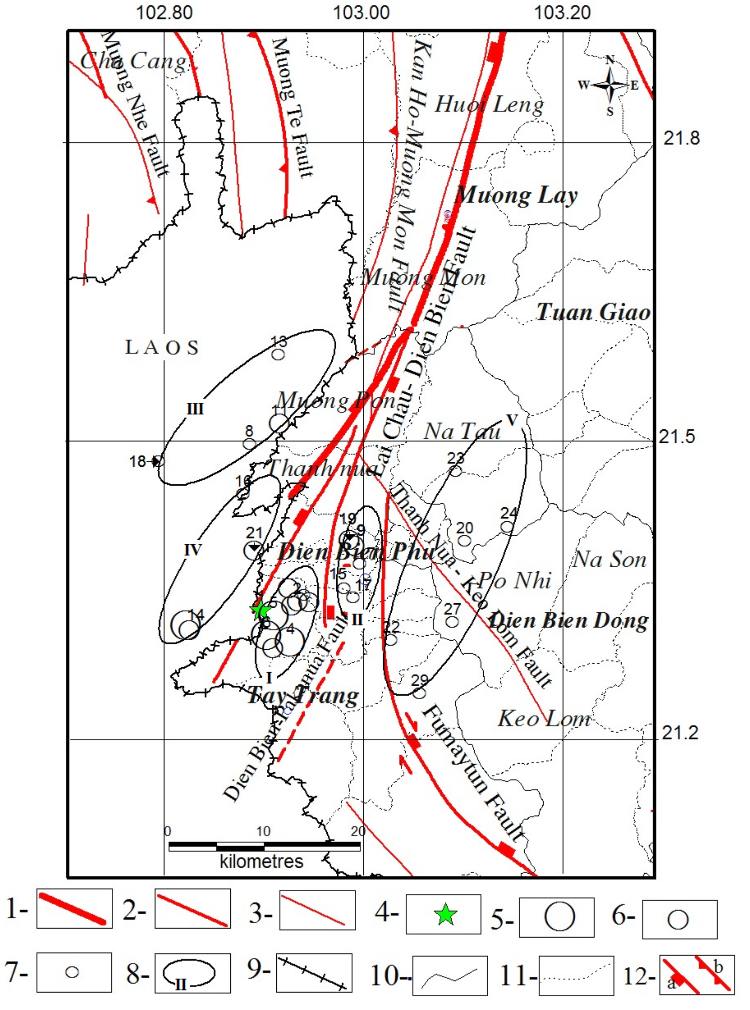

Fig. 1. Spatial distribution and temporal evolution of aftershocks.
Notes: 1 – first-order fault; 2 – second-order fault; 3 – undivided high- order fault; 4 – mainshock; 5 – 4.0 ≤ M < 5.0; 6 – 3.0 ≤ M < 4.0; 7 – M < 3.0; 8 – distribution area of aftershock series (I–V); 9 – Vietnam's border; 10 – district boundary; 11 – commune boundary; 12a – normal fault; 12b – reverse fault (tectonic faults after Nguyen Ngoc Thuy et al., 2005).

of 53 km^2 with a width of 5 km and an axis of 10 km in the NE–SW direction, coinciding with the direction of LC-DB fault. Thus, the dimension and area of distribution of the first aftershock series are relatively consistent with the dimension of hypocentre of mainshock presented in Table 1 (Shebalin, 1971, 1974). It means that the first aftershock series was located in the extremely active zone, which was the source zone of Dien Bien earthquake. It can be observed from Fig. 1 and Table 1 that about 15 min after the mainshock, an aftershock with a magnitude of M = 3.1 occurred immediately (aftershock 1); its epicentre was located 25 m slightly eastwards compared to the mainshock and on the right of LC-DB fault in the first aftershock series. The subsequent aftershocks also shifted towards this direction compared to the mainshock (aftershocks 2, 3, 4, 6 and 7) and were distributed quite concentratedly in the first aftershock series. The epicentre of the strongest aftershock with a magnitude of M = 4.8 was also distributed in this series (aftershock 4).

- ***The second aftershock series (II):*** This series consists mainly of the aftershocks that followed the aftershocks of the first series. Their epicentres were also distributed concentratedly in a smaller ellipse-shaped area of about 44 km^2 with a width of 4.4 km and an axis of nearly 13 km in the sub-longitudinal direction and more northwards compared to the mainshock. It is noteworthy that the second aftershock series lay between two faults towards the right of LC-DB fault and the left of Fumaytun fault. Both segments of these two faults follow the sub-longitudinal direction, coinciding with the extending direction of the second aftershock series.

- ***The third aftershock series (III):*** This series consists of the aftershocks occurring later than those of the second series with smaller magnitude M. They were extensively distributed in an ellipse-shaped area of about 148 km^2 (with a length of 24 km and a width of 7.9 km) towards the left of LC-DB fault and more towards northwest direction compared to the mainshock and the first two aftershock series. Particularly, the third aftershock series seemed to be distributed closer and located at the end of Kan Ho-Muong Mon fault.

- ***The fourth aftershock series (IV):*** This series includes the aftershocks that followed the aftershocks of the third series. Their epicentres were distributed on the left of LC-DB fault branch and more towards northwest direction compared to the mainshock, and more towards southeast direction compared to the third aftershock series. They were also distributed in a smaller ellipse-shaped region compared to the third series with a length of about 21.5 km, a width of about 6.1 km, an area of 106 km^2 and extended in the NE–SW direction, coinciding with the extending direction of the LC-DB fault.

- ***The fifth aftershock series (V):*** This series includes the latest aftershocks (with smaller magnitude M) that were distributed outwards on the right of both LC-DB fault and Fumaytun fault. This series shifted more towards north direction compared to the mainshock and the first aftershock series. They were concentrated in the largest ellipse-shaped area of 239 km^2 with a width of about 9.4 km and an axis of 33 km in the NE–SW direction, coinciding with the extending direction of LC-DB fault. It is noteworthy that the fifth aftershock series was distributed furthest from LC-DB fault but closer to Fumaytun fault and lay on both sides of Thanh Nua-Keo Lom fault branch.

Thus, the above-described process has shown that the development in the hypocentre region (source zone) of Dien Bien earthquake consists of five aftershock series with the evolution recurring on both sides of LC-DB fault and shifting towards different directions compared to the mainshock: After the mainshock, the first aftershock series occurred on the right side of LC-DB fault in the Dien Bien Phu segment and then the aftershock activities gradually shifted towards east and NE directions on the right of this fault. Subsequently, the aftershock activities developed on the left of LC-DB fault and shifted towards northwest direction, then slightly towards SW direction and finally extended on the right of LC-DB fault towards the east–NE direction compared to the mainshock. This process is the evidence that not only indicates the consistency of seismic and tectonic data but also confirms the active and recent activities of LC-DB fault and other faults in this area. It is also the cause of Dien Bien earthquake and its aftershock series.

Depth distribution of aftershocks

The depth distribution of hypocentres of mainshock and its aftershocks is presented in Fig. 2. It is clear that the hypocentre of Dien Bien earthquake was located at a depth of 6.1 km and the hypocentres of its aftershocks were located at a depth of 0–10 km, mainly concentrated on the surface (Fig. 2, Table 1). This result shows that Dien Bien earthquake is a shallow earthquake, with the hypocentre located near the Earth's surface.

Relation between characteristics of aftershock activities of Dien Bien earthquake and morphotectonic features in the area

To determine the relationship between characteristics of the aftershock activities of Dien Bien earthquake and geomorphic features caused by tectonic activities in the studied area, we have established the distribution diagram of epicentre of Dien Bien earthquake and its aftershocks based on the digital elevation map (Fig. 3) and distribution map of morphotectonic indicator (V_f index) and lineament density (Fig. 4).

On the basis of the results obtained from Figs 3 and 4, the spatial distribution and temporal evolution of aftershock series of the Dien Bien earthquake have been examined together with topographic and morphotectonic analysis. It can be observed that all five aftershock series were distributed along deep

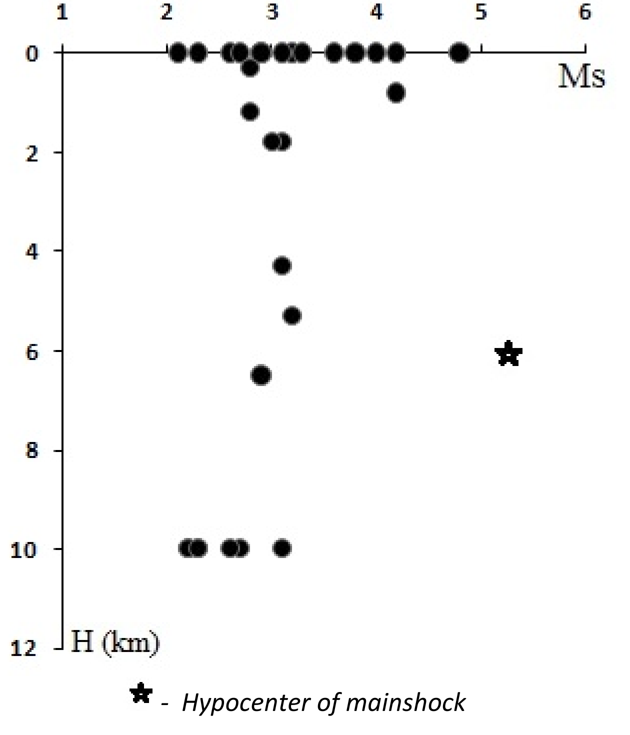

Fig. 2. Depth distribution of hypocenters of Dien Bien earthquake and its aftershocks.

valleys or steep slopes belong to tectonic fault branches (Fig. 3). The aftershock series with greater magnitude and earlier occurrence time were distributed in deep valleys or their slopes (the first and second series). The weaker and later aftershock series were distributed along steep slopes (the third, fourth and fifth series). It allows us to comment that the active activities of LC-DB fault along with its neighbouring fault branches caused the destruction and movement of matter in the existing geological environment and triggered Dien Bien earthquake and its aftershocks. The occurrence of aftershock series and their magnitude do not only depend on the distance from the mainshock and active faults that cause them but also on the terrain elevation, where they occurred.

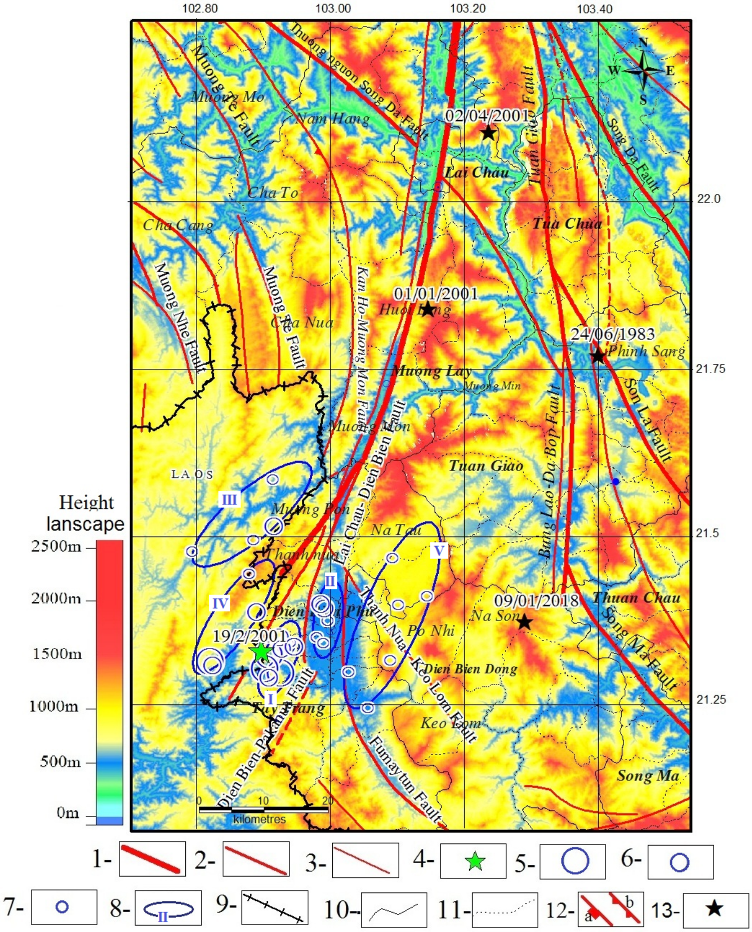

Fig. 3. The characteristics of aftershocks of the Dien Bien earthquake on the digital elevation map.

It should be noted that the legends of Fig. 3 are similar to those of Fig. 1; 13 in the figure represents the epicentre of the earthquakes, corresponding in Table 2.

Comparison of the aftershock series distribution of the Dien Bien earthquake with distribution of the geomorphic index (V_f) and the lineament density on Fig. 4 shows that all five aftershock series are distributed where the geomorphology anomalies are quite high. In particular, the aftershock series with greater magnitude and earlier occurrence time (the first and second series) are distributed in the central of Dien Bien Phu basin, where the highest value of geomorphic index (V_f = 2.2–4.0), and the lineament density is also relatively high (about 0.35–0.5m/km^2). The weaker and latter aftershock series (the third, fourth and fifth

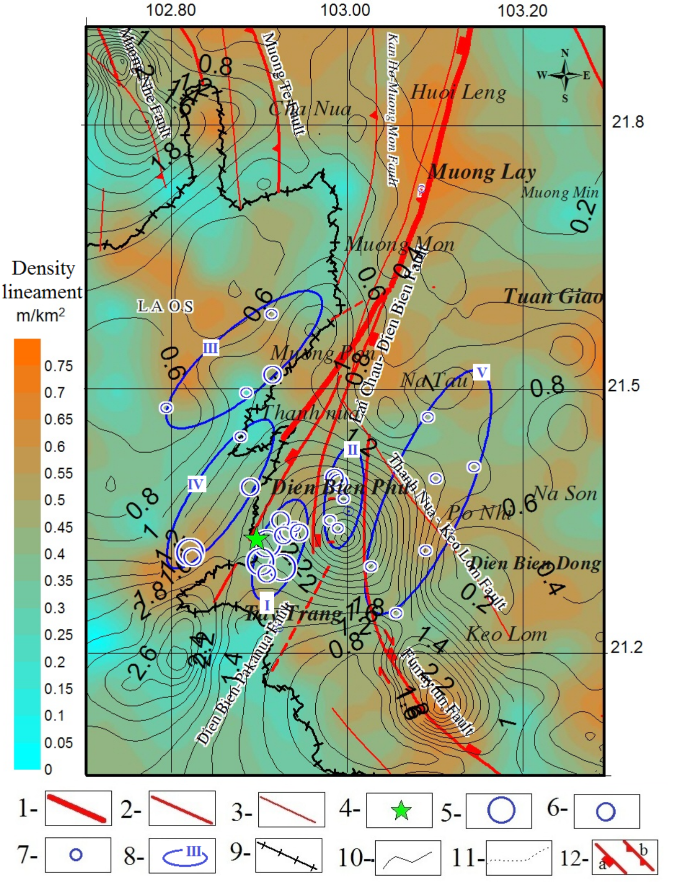

Fig. 4. Relation between characteristics of aftershocks of Dien Bien earthquake and morphotectonic indicator (V_f) and lineament density in the studied area.

Notes: 1–9 – are similar to those of 1–9 in Fig. 1; 10 – isoline of geomorphic index value V_f; 11a – normal fault; 11b – reverse fault.

series) were distributed at the area with lower values (V_f = 0.4–1.4). However, here the lineament density is higher (approximately 0.55–0.7 m/km^2) (Fig. 4).

Thus, the temporal evolution (early or late) and magnitude of aftershock series depend not only on the closer or further distance compared to the mainshock and the active faults that cause them but also on terrain elevation, slope index, lineament density and their positions relative to other tectonic faults in the studied area.

According to the results from the combination of different studying methods such as analysing morphotectonic indicators, age dating of young terraces as well as precise geodetic measurements (Zuchiewicz, Cuong, 2002, 2003; Zuchiewicz et al., 2004), the LC-DB fault zone is a sinistral, sinistral-normal one. It does not only activate with rate of sinistral strike-slip ranging from 2 to 4 mm/yr in middle-late Pleistocene and from 0.6 to 2 mm/yr in Holocene and also about 1 mm/yr at present. The rate of Holocene uplifting is about 0.4–0.6 mm/yr in the western part of Dien Bien Phu, exactly where the Dien Bien Earthquake took place on 19 February 2001.

The activities of the LC-DB fault zone are more intensive on the radiating fault branches that develop to both two sides in its SW (Thanh Nua-Keo Lom, Fumaytun, Dien Bien-Pakanua, Kan Ho-Muong Mon faults), consistent with the locations where the aftershocks occurred.

If applying a larger scale to examine Dien Bien earthquake and its aftershocks along with other earthquakes in Dien Bien province (Fig. 3), Tuan Giao earthquake on 24 June 1983 (M = 6.7), Lai Chau 1 earthquake on 1 January 2001 (M = 4.5), Lai Chau 2 earthquake on 2 April 2001 (M = 4.9), and the latest Dien Bien 2 earthquake on 9 January 2015 (M = 4.7) (Table 2), it can be observed that amongst the five earthquakes occurring from 1983 to present, three earthquakes occurred along LC-DB fault at different segments: Lai Chau 1, Lai Chau 2 and Dien Bien 1. Their epicentres were distributed in a strip extending in NE–SW direction, and their occurrence repeated in this direction. Lai Chau 1 earthquake occurred earliest on LC-DB fault at Lai Chau segment, followed by Dien Bien 1 earthquake on this fault at Dien Bien. Its epicentre shifted more towards SW direction compared to Lai Chau 1 earthquake. Subsequently, Lai Chau 2 earthquake also occurred on the right of this fault and its epicentre shifted more towards NE direction compared to two above-mentioned earthquakes. Thus, the seismicity in LC-DB area is quite active and the earthquake activities occur repeatedly along LC-DB fault. This process is the evidence that confirms the active and recent activities of LC-DB fault.

The results obtained is very important and seems to open a new approach in the study of earthquake activity characteristics, especially in active seismic regions based on the use of combinations of seismic, tectonic, geomorphic and remote sensing data.

T a b l e 2. Parameters of some earthquakes occurring in Dien Bien province.

No.	Earthquake	Date	h	m	s	ϕ^o N	λ^oE	H (km)	M
1	Tuan Giao	24.06.1983	14	18	22.3	21.77	103.4	23	6.7
2	Lai Chau 1	01.01.2001	11	34	21.1	21.84	103.146	1.1	4.5
3	Dien Bien 1	19.02.2001	15	51	35.2	21.331	102.897	6.1	5.3
4	Lai Chau 2	02.04.2001	20	45	48	22.103	103.236	0.1	4.9
5	Dien Bien 2	08.01.2018	23	21	21.8	21.374	103.29	10	4.7

Conclusion

The characteristics of Dien Bien earthquake on 19 February 2001 and its aftershocks have been studied and examined in detail. On the basis of the spatial distribution and temporal evolution of aftershock series of Dien Bien earthquake, its development in the source zone has been clarified, which consists of five aftershock series recurring on both sides of LC-DB fault and shifting towards different directions compared to the mainshock. This process is the evidence that not only indicates the consistency of seismic and tectonic data but also confirms the active and recent activities of LC-DB fault and other ones in this area. It is also the cause of Dien Bien earthquake and its aftershocks.

Examining the characteristics of aftershock activities of Dien Bien earthquake in relation to geomorphological features and tectonics activities in the area reveals that the magnitude of aftershock series and their temporal evolution (early or late) depend not only on the closer or further distance compared to the mainshock and the active faults that cause them but also on terrain elevation, slope index, lineament density and their positions relative to other tectonic faults in the studied area. Therefore, it is necessary to study in detail other earthquakes and their aftershocks in relation to geomorphological and tectonic features in order to clarify the mechanism and serve the prediction of aftershocks following the mainshock.

Acknowledgements

The authors would like to thank the support of the scientific research mission in 2018–2019 (Code: NVCC12.01/18-18; NVCC12.01/19-19).

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