EFFECT OF INVASIVE SPECIES *IMPATIENS PARVIFLORA* ON SOIL MICROBIAL INDICES IN THE PROTECTED AREAS IN SLOVAKIA

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Abstract

Biological invasions are one of the main threats to natural ecosystems and the impact of invasive plant species on native species, communities, ecosystems and soil biota has been widely recognized over the last decades. Costs of invasive species are estimated to range from millions to billions of euros annually and the success of invasive species has been attributed to their biological and ecological trails. Our study aimed to assess the effect of invasive plant species *Impatiens parviflora* on selected soil microbial indices and physicochemical characteristics. The research was carried out during a vegetation season on 3 protected areas of Norths-Eastern Slovakia in 2015. Soil reaction, soil organic carbon, bulk density, soil porosity and soil moisture were selected to determine soil physicochemical properties. As the microbial indices, that mainly indicate soil microbial activity, we selected soil microbial respiration and soil enzymatic activity (urease, acid and alkaline phosphatases). The results showed that *I. parviflora* prefers acidic and non-compacted soil conditions. The results also suggested that biology of the invasive plants had the high impact on soil ecosystem and soil enzyme activity played an important role in nutrient cycling in the ecosystems, and thus could be considered as biological indicator of soil health and environmental changes.

Keywords: *Impatiens parviflora, Enzymatic activity, Protected areas, Soil characteristics.*

Introduction

Invasive plants dramatically influence biodiversity in non-native areas and often create monocultures where diverse plant communities previously existed (Zhang *et al.,* 2009). Changes in the plant species composition might result in changes in the enzymatic activities of the microbiota. Such changes could be particularly important in plant communities that are heavily invaded by exotic species. Invasion of those species can alter many ecosystem properties, including important soil function and characteristics (Kourtev *et al.,* 2002). Therefore, the documented impacts on soil properties are diverse. In addition, the same species may have different impacts, depending on local conditions (Dassonville *et al.*, 2008). In general, exotic invasive species have higher net primary productivity and higher turnover rates of carbon and nitrogen, but the opposite pattern was also found. These impacts are also mediated by alteration in soil microbial communities (Herr *et al.*, 2009, Kourtev *et al.*, 2002). Moreover, one of the mechanisms of invasive species success is the production and release of allelopathic compounds by invader that are harmful to plant neighbours (Baležentienė and Renčo, 2014).

Characterization of soil state may be mediated by physical and biochemical parameters related to certain soil processes and functions. Monitoring of soil quality and health relies on the use of specific indicators. Mostly biochemical properties are considered to be the best indicators for assessing soil quality, but their use is limited by the lack of available data

(Doran and Zeiss 2000; Miralles *et al.*, 2007). Soil microbial activities, including enzyme activities, are more sensitive compared to physical and chemical properties to changes in soil quality and can be easily quantified. Soil biochemical properties are regarded as relevant indicators of soil quality from an environmental viewpoint and could be useful for monitoring changes in many type of ecosystems (Kizilkaya *et al.*, 2004; Nowak and Schneider, 2017).
The aim of this study is to assess the effect of invasive plant species *I. parviflora* on selected soil physicochemical characteristics and soil microbial indices.

Material and Methods

Site description

The research was carried out during a vegetation season (July 2015) in 3 protected areas of North-Eastern Slovakia - National Nature Reserve (NNR) Šarišský hradný vrch, National Nature Reserve (NNR) Kokošovská dubina and Nature Reserve (NR) Fintické svahy in 2015. One native site, without *I. parviflora*, was also included for comparative purpose (as Control site). This region is known for its high diversity of animal and plant life, and all these forms are highly responsive to environmental changes. Mostly moderate Cambisols are typical in all researched areas in warm, medium wet climatic regions with the cold winter. The soil was sampled in three different microhabitats - meadows (a habitat without tree vegetation), a habitat close to the stumps (as the bottom part of a tree left) and a habitat under dense tree vegetation, in each natural reserve. Soil samples were taken in triplicates from all the microsites to ensure high homogenity of the measured data. Obtained data were tested by mathematical-statistical methods from which analysis of variance and regression analysis were used (the Statgraphics software package).

Soil physicochemical analysis

Air-dried soil samples were used to measure soil pH and organic carbon content. Gravimetric soil moisture was calculated on 10g of fresh subsamples after drying in a 105 °C oven for 24 hours. Soil pH was detected in a 1:3 mixture of soil and 0.01M $CaCl_2$ solution using a digital pH meter. Soil organic carbon (SOC) was determined by the Turin's method. Soil bulk density [$t.m^{-3}$] and porosity [%] were evaluated in a Kopecky physical cylinder with a capacity of 100 cm^3 (Fiala et al. 1999).

Soil respiration and enzyme assays

Soil microbial respiration was (SMR) measured by the CO_2 released from 100g samples of field moist soil in 500 mL hermetically sealed flasks at 25 °C for 14 days. CO_2 was captured by its reaction with NaOH (1 mol L^{-1}) and titrated with HCl (0.5 mol L^{-1}) after the addition of $BaCl_2$ (1 mol L^{-1}). The concentration of the released carbon was estimated in μg C-CO_2 g soil dry^{-1} day^{-1} (Alef and Nannipieri 1995). Enzymatic activity assays were determined using previously selected analysis of field-moist soil samples, which were sieved and properly homogenized. Activity of acid and alkaline phosphatase was determined by Grejtovský (1991) and urease activity by Khaziev (1976).

Results and Discussion

All research sites were located in the forest biotopes that naturally give the ecosystem acidic soil reaction. Lower pH was observed in NR Fintické svahy, as well as organic carbon content. All determined soil physical and chemical average parameters are referred in Table 1.

Soil pH is an important factor of soil health despite of the fact that its value changes under internal and external factors. Jurko (1990) and Chmura (2006) confirmed that *I. parviflora* prefers acidic soil reaction, which was also shown in our study. Chmura (2006) also found out the highest influence of chemical features (organic carbon and nitrogen content) of species *I. parviflora* near the stump habitats. Soil physicochemical characteristics are indicators of soil ability to absorb and maintain soil water. Soil bulk density, porosity, soil moisture, organic matter and soil reaction are considered as ones of the basics soil physical and chemical properties (Fazekašová *et al*., 2011). Soil moisture is considered to be one of the most important factors affecting soil respiration, but our study did not show this significant relation which was also found in the study of Bobuľská et al. (2015). The highest soil moisture was also measured in NR Fintické svahy, especially in the stump habitats. Coombe (1956) states that *I. parviflora* grows on non-compacted and structured soils maintained high humidity, except of flooded localities. Bulk density is closely connected to the total soil porosity. In NR Fintické svahy, the presented research encountered both, the highest porosity and the lowest bulk density in soil. The previous studies also proved that the abundance and biological characteristics of *I. parviflora* also depends on soil physicochemical properties (Bobuľská *et al*., 2016), but our study also confirmed that soil microbial activity is very important factor in characterization of soil state.

Table 1. Soil physicochemical characteristics of three microhabitats in nature reserves

Natural reserves	Microhabitats	pH/CaCl2	Bulk density [t.m-3]	Porosity [%]	Soil moisture [%]	SOC [%]
NNR Šarišský hradný vrch	Meadow	6.7	1.33	49.81	26.2	5.13
	Habitat under tree vegetation	6.5	1.15	56.60	35.8	5.08
	Stumps habitat	7.1	0.94	64.53	34.9	4.99
NNR Kokošovská dubina	Meadow	6.2	1.12	57.54	30.7	5.20
	Habitat under tree vegetation	7.0	0.95	64.15	35.8	5.12
	Stumps habitat	6.2	1.11	58.11	36.9	5.08
NR Fintické svahy	Meadow	5.9	0.86	67.55	33.0	5.15
	Habitat under tree vegetation	5.8	0.87	67.17	40.3	5.02
	Stumps habitat	5.4	0.62	76.60	54.8	4,88
Control site		7.2	1.09	49.96	31.5	5.98

Determination of the intensity of microorganisms' respiration is of great importance. It refers not only to the overall biological activity of the soil, but also the speed of mineralization processes. In addition to temperature and humidity, soil respiration isinfluenced by the quality and supply of nutrients in the soil, soil texture, soil aeration, type of ecosystem, and increasingly also by soil managements. In general, invasive species might increase the activity of microbial parameters, as well as the functional diversity of microbial communities with the significant carbohydrate utilization (Zhang *et al*., 2009) that characterize stress of the bacterial

communities due to poor nutrient status (Pessi *et al*., 2012). Also in our study, the sites with the invasive species showed higher values of microbial parameters compared to the control sites (*p<0.05). Average values of the selected soil biological (microbial) parameters, such as soil microbial respiration (SMR) and soil enzymatic activities, are given in Table 2. Soil basal respiration was higher under the stamps habitat that was represented by the highest plant individuals with the most number of flowers/fruits as it was mentioned in the previous biological study of *I. parviflora* (Bobuľská *et al*., 2016). Castillo and Joergensen (2001) pointed out that almost all biological properties are significantly influenced by environmental management, of which the soil respiration was the parameter most affected by way of different management and many other factors. Same trend was also observed with the soil urease activity that tends to increase the pH of the environment as it produce ammonia as a basic molecule (Monreal and Bergstrom 2000). There are a number of enzymes in soil, depending on diversity of soil organisms and conditions of organic substances turnover. Several factors may affect directly or indirectly the activities of soil enzymes (Okur *et al*., 2010). Soil reaction differs from the pH optimum for phosphatase activity. Soil phosphatase activity is higher in soils with high humidity and because phosphatases have different optimal pH, therefore are divided into acid and alkaline phosphatases (Speir et al., 2003). Phosphatases are produced not only by the microorganisms, but also by higher plants. In our study, activity of acid phosphatase was higher compared to alkaline phosphatase activity in all localities (**p<0.01), which was closely connected to acidic condition on the research sites. Also the activity of acid phosphatase was significantly higher (*p<0.05) in the microhabitat under tree vegetation. The high activity of this enzyme may originate from roots of such higher plants (Senga *et al*., 2011).

Table 2. Soil microbial characteristics of three microhabitats in nature reserves

Natural reserves	Microhabitats	SMR [μg C-CO_2/g soil]	Urease [mgNH_4^+-N g^{-1} d^{-1}]	Acid Phosphatase [mg P g^{-1} $3h^{-1}$]	Alkaline Phosphatase [mg P g^{-1} $3h^{-1}$]
NNR Šarišský hradný vrch	Meadow	103.55	0.43	377.81	266.23
	Habitat under tree vegetation	127.85	0.55	466.60	305.56
	Stumps habitat	172.05	0.74	394.53	340.09
NNR Kokošovská dubina	Meadow	105.67	0.42	588.54	400.75
	Habitat under tree vegetation	120.41	0.49	603.15	405.83
	Stumps habitat	135.45	0.80	599.11	389.93
NR Fintické svahy	Meadow	112.08	0.52	467.55	334.06
	Habitat under tree vegetation	125.46	0.63	527.17	406.31
	Stumps habitat	129.89	0.92	516.60	440.18
Control Site		100.88	0.41	237.99	250.65

Conclusion

Soil microbial indices, as well as biochemical are sensitive to not only under the influence management changes, but under the environmental changes. Our findings showed that *I. parviflora*, as invasive plant species, prefers acidic and non-compacted soil conditions. The results also suggested that biology of the invasive plants had the high impact on soil ecosystem and soil enzyme activity played an important role in nutrient cycling in the ecosystems, and thus could be considered as biological indicator of soil health and environmental changes.

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