Czech University of Life Sciences Prague
Faculty of Environmental Sciences

FACULTY OF ENVIRONMENTAL SCIENCES

DEPARTMENT OF ECOLOGY

CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

The effect of long-term fertilizers application on soil chemical properties, plant species composition and arbuscular mycorrhizal fungi of grasslands

(Vliv dlouhodobého hnojení na chemické vlastnosti půdy, rostlinné společenstvo a arbuskulární mykorhizní houby v travních společenstvech)

Thesis extended summary

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Doktorská disertační práce „The effect of long-term fertilizers application on soil chemical properties, plant species composition and arbuscular mycorrhizal fungi of grasslands (Vliv dlouhodobého hnojení na chemické vlastnosti půdy, rostlinné společenstvo a arbuskulární mykorhizní houby v travních společenstvech)“ byla vypracována v rámci doktorského studia na Katedře ekologie Fakulty životního prostředí České zemědělské univerzity v Praze.

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S disertační prací je možné se seznámit na Oddělení pro vědu a výzkum FŽP ČZU v Praze, Kamýcká 129, Praha 6 – Suchdol.

CONTENT

1.1 GENERAL INTRODUCTION

With increasing usage of mineral fertilizers at the beginning of the last century, there has also been a higher demand for a more multidisciplinary and holistic contribution to the agronomic research and development (Conway, 1985; Schellberg *et al*., 1999). Agroecosystems provide high variety of valuable services such as production of food, fiber, fuel and many others. However human activities linked with more intensive land use have also serious negative consequences such as chemical contamination of the soil, water and air, decrease of biodiversity, soil erosion, alteration of the most important biogeochemical cycles and others. Nonnegligible portion of these changes is caused by fertilization (Wood *et al*., 2000; Schröder *et al*., 2008).

Fertilization and liming have been used as an instrument improving hay production for centuries (Hejcman *et al*., 2013; Kennedy *et al*., 2005). For a long period of time, these practices not only sustained and increased hay yields, but also had negative effects on grassland ecosystems (Schellberg *et al*., 1999; Egerton-Warburton *et al*., 2007). Among others, they caused considerable changes in plant species composition leading in most cases to the reduction of species richness (**Paper I**) (Silvertown *et al*., 2006; Kennedy *et al*., 2005) due to a lower capability of indigenous plants to compete with more productive pasture species (Schellberg *et al*., 1999). Furthermore these practices caused changes in soil and biomass chemical properties and shifts of soil microbial communities, including arbuscular mycorrhizal fungi (Hejcman *et al*., 2010; Johnson *et al*., 2010) (**Paper II**).

Arbuscular mycorrhizal fungi establish association with plant roots mutually beneficial for both partners where plant hosts provide carbohydrates in exchange for mineral nutrients, water or other provision from the fungi (Allen, 1991; Jansa *et al*., 2003; Parniske, 2008). They are very important components of the soil microbial community forming symbioses with more than two thirds of terrestrial plant species (Smith and Read, 2008; van der Heiden, 1998; Bonfante and Genre, 2010). In addition to the improvement of plant nutrient uptake, arbuscular mycorrhizal fungi can provide better drought tolerance or resistance against pathogens for the host (Newsham *et al*., 1995; Smith *et al*., 2010) and their hyphae form an underground network in the soil which helps to create stability of soil aggregates and reduce soil erosion (Gryndler *et al*., 2004; Chaudhary *et al*., 2009). It has been shown that more diverse arbuscular mycorrhizal fungi community significantly increases species richness and productivity of plant communities (Johnson, 1993; van der Heijden *et al*., 1998; Liu *et al*., 2012). Consequently, any changes altering community composition of arbuscular mycorrhizal fungi can change function of plant community (Johnson, 1993; Corkidi *et al*., 2002; Johnson *et al*., 2003). Arbuscular mycorrhizal fungi thus create a pivotal link between plants and soil and they are essential parts of grasslands (Grime *et al*., 1987; Gange *et al*., 1990; Sanders and Koide, 1994).

To overcome the problems related to eutrophication or intensification caused by long-term fertilization, professional knowledge of the effects of soil nutrient supply is necessary **(Paper III)**. For these proposes, in the middle of the 19th century (after the industrial production of fertilizers), the long-term experiments were set up to contribute the research at the level of botany, soil, microbiology, agriculture or landscape ecology. Long-term studies represent a perfect source providing knowledge on complex interactions between fertilizer input, plant species composition, soil chemical properties, productivity, forage quality and soil biota including mycorrhizal fungi (Schellberg *et al*., 1999; Liu *et al*., 2012). Some effects are only noticeable after a longer period of time and long-term experiments can help to avoid the difficulties which can occur due to the seasonal variation in growing conditions. By recognition of all impacts and ecosystem functioning, grasslands or other agroecosystems protection would be more effective (Thurston *et al*., 1976; Liu *et al*., 2012).

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CHAPTER 2

2.1 AIMS AND HYPOTHESES OF THE THESIS

The aim of the dissertation thesis was to investigate the effect of fertilizer application on plant and mycorrhizal community and soil chemical properties in grassland ecosystems and to further examine how the different nutrient enrichment changes plant species composition and production, soil and biomass chemical properties, community of arbuscular mycorrhizal fungi, their presence, abundance and other parameters. This thesis was divided into three specific aims:

Specific aims:

2.1.1 The Steinach Grassland Experiment: Soil chemical properties, sward height and plant species composition in three cut alluvial meadow after decades-long fertilizer application

The aim of this experiment was to analyze the effect of nitrogen, phosphorus, potassium, and calcium application on biomass and soil chemical properties, compressed sward height, and plant species composition in Steinach Grassland Experiment, probably the oldest still-running grassland fertilizer experiment in Continental Europe.

H_1: Decades-long fertilization with nitrogen, phosphorus, and potassium will change soil chemical properties (in particular soil pH, total nitrogen, organic carbon and plant available concentration of phosphorus, potassium and calcium in the soil).

H_2: Nutrients addition (either alone or in combination) will affect productivity of the vegetation estimated using compressed sward height.

H_3: Enrichment with nitrogen, phosphorus, potassium, and calcium will change plant species composition of the sward.

H_4: Nitrogen enrichment will reduce species richness of vascular plants.

2.1.2 Long-term agricultural management maximizing hay production can significantly reduce belowground carbon storage

The aim of this study was to investigate the responses of soil carbon and arbuscular mycorrhizal fungi to seventy years of application of lime and mineral fertilizer. The goal of this work was to predict responses of soil carbon stocks to grassland inputs. We examined the abundance of arbuscular mycorrhizal fungi inside and outside plant roots and used molecular techniques to determine the abundance of a few individual arbuscular mycorrhizal fungal taxa.

H_1: Lime and mineral fertilizers (nitrogen and phosphorus) will reduce plant allocation of photosynthates to belowground structures including the arbuscular mycorrhizas.

H_2: Differences in lime and fertilizer input among treatments will affect long-term buildup of soil carbon stocks, co-incident with the effects on arbuscular mycorrhizal fungi.

H_3: Nitrogen enrichment will increase allocation to arbuscular mycorrhizal fungi.

H_4: Fertilization with nitrogen and phosphorus will decrease allocation to arbuscular mycorrhizal fungi, consequently reducing the soil carbon sequestration.

2.1.3 *Zea mays* responds to 70 years of differently managed soils

The aim of this experiment was to examine the responses of investigated plant *Zea mays* (height of plant, number of live and dead leaves, dry plant above-ground biomass, flowering occurrence), grown on soils from long-term experiment Rengen Grassland Experiment which differ in soil chemical parameters affected by long-term fertilization of nitrogen, phosphorus, and calcium.

H_1: Enrichment with nitrogen, phosphorus and calcium will have significant effects on soil chemical properties.

H_2: Soil chemical properties (pH, content of nutrients in the soil) will significantly affect morphologic and qualitative parameters of investigated plants.

H_3: Fertilization with more nutrients will result in higher height of plants.

H_4: Soil nutrient addition will increase dry plant above-ground biomass and number of leaves of the plant.

CHAPTER 3

The Steinach Grassland Experiment: Soil chemical properties, sward height and plant species composition in three cut alluvial meadow after decades-long fertilizer application

Abstract

The Steinach Grassland Experiment (SGE) is probably the oldest still running grassland fertilizer experiment in Continental Europe. It has been established on an alluvial *Alopecurus pratensis* meadow in SE Germany in 1933. The aim of this paper was to provide for the first time detailed information on this experiment about effects of decades-long nitrogen, phosphorus, potassium, and calcium application (46 treatments altogether) on (1) soil chemical properties, (2) sward height, (3) plant species composition, and (4) species richness of vascular plants derived from a field survey in spring 2008.

(1) A steep gradient of soil properties was recorded, namely plant available (Mehlich III) P (14–161 mg kg-1), K (82–1018 mg kg-1) and Ca (532–3336 mg kg-1) concentrations, as well as C:N ratio (6.9–10.4), and pH (H2O) (4.7–7.0). (2) Compressed sward height in the third week of May 2008 ranged from 11 cm in the control to 47 cm in the limed plot with high N, P, and K application.

(3) Although fertilizer application changed plant species composition, diversification of plant communities was not as high as in the case of fertilizer application on naturally low productive soils in other well-known long-term experiments. This was given by sufficient nutrients enabling survival of species adapted to high nutrient availability even in the unfertilized control. This indicates that species composition in productive alluvial grasslands is substantially less affected by fertilizer application than in low productive grasslands.

(4) Long-term fertilizer application negatively affected plant species richness of vascular plants directly by soil acidification and indirectly by an increase in sward height. The extent to which N application negatively affected species richness was dependent on whether N was applied alone or in combination with other nutrients and whether the N application acidified the soil or not.

Citation: Hejcman M., Sochorová L., Pavlů V., Štrobach J., Diepolder M., Schellberg J. (2014): The Steinach Grassland Experiment: Soil chemical properties, sward height and plant species composition in three cut alluvial meadow after decades-long fertilizer application. Agriculture, Ecosystems and Environment (184): 76 – 87.

Authorship: Hejcman M., Sochorová L., Pavlů V., Štrobach J., Diepolder M., Schellberg J.

Keywords: fertilization; long-term experiment; nitrogen, phosphorus, potassium, and calcium; plant species richness and diversity; sward height

Long-term agricultural management maximizing hay production can significantly reduce belowground C storage

Abstract

Liming and fertilization of grasslands have been used for centuries to sustain hay production. Besides improving hay yields, these practices induce compositional shifts in plant and soil microbial communities, including symbiotic arbuscular mycorrhizal (AM) fungi. However, in spite of increasing interest in soil carbon (C) sequestration to offset anthropogenic CO_2 emissions, little is known about the long-term effects of these agronomic interventions on soil C stocks. We examined how plants, AM fungi, and soil C respond to more than seven decades of annual applications of lime, mineral nitrogen (N), and mineral phosphorus (P) to test the hypotheses that (1) management practices increasing aboveground plant production decrease C allocation to roots, AM fungi and the soil; and (2) the relative availability of N and P predicts belowground C allocation in a consistent manner. Our study was conducted at the Rengen Grassland Experiment, established in 1941. Lime combined with N increased hay yields and promoted development of AM fungal hyphae in soil, while reducing relative C allocation to roots. Simultaneous enrichment of soil with lime, N, and P further boosted hay production, promoted grasses and suppressed other plant functional groups. This treatment also decreased soil organic C and strongly suppressed AM fungi in the soil, although the response to P varied among different AM fungal taxa. Our results indicate that agricultural management practices aimed at maximization of hay production may, in the long run, significantly (-20%) reduce belowground C storage. This is a great concern with respect to the intended use of grasslands as anthropogenic CO_2 sinks because the fertilization-induced decrease in soil C stocks can partly or fully negate the C sequestration potential of the grassland ecosystems as a whole.

Citation: Sochorová L., Jansa J., Verbruggen E., Hejcman M., Schellberg J., Kiers E.T., Johnson N.C. (2016): Long-term agricultural management maximizing hay production can significantly reduce belowground C storage. Agriculture, Ecosystems and Environment (220): 104 – 114.

Authorship: Sochorová L., Jansa J., Verbruggen E., Hejcman M., Schellberg J., Kiers E.T., Johnson N.C.

Keywords: arbuscular mycorrhiza, nitrogen, phosphorus, lime, soil carbon, long term ecological research

CHAPTER 5

Zea mays responds to 70 years of differently managed soils

Abstract

This study has shown that seventy years of different fertilizer treatment at the Rengen Grassland Experiment (RGE) caused changes in soil chemical properties which have resulted in significantly various plant responses. The goal of this study was to examine the responses of *Zea mays* to the soils collected from the RGE and grown in greenhouse. In June 2012, soil from four fertilizer treatments (only limed, limed with nitrogen, limed with nitrogen and phosphorus, and unfertilized control without any nutrient input) was collected in RGE and used to plant germinated seedling of *Zea mays* for nine weeks. Once a week, height of plant, number of leaves was counted and dry above-ground biomass was weighted at the end of experiment.

We found that long-term fertilization and liming caused high variation in soil pH, plant available concentration of phosphorus and organic carbon in the soil but it did not significantly altered total content of nitrogen and plant available calcium in the soil. Also, height of plants varied markedly across the treatment with the highest values in limed soils enriched with nitrogen. The same trend was observed for number of leaves and dry above-ground biomass.

Citation: Zemková L. (2016): *Zea mays* respons to 70 years of differently managed soils. Scientia Agriculturae Bohemica (Submitted paper).

Authorship: Zemková L.

Keywords: bioassay, *Zea mays*, nitrogen, phosphorus, calcium, long term grassland experiment

SOUHRN (SUMMARY IN CZECH)

Tato disertační práce je zaměřena na zkoumání vlivu dlouhodobého hnojení na rostlinné společenstvo, půdní parametry a společenstvo mykorhizních hub v travních ekosystémech a to konkrétně vliv jednotlivých živin (dusík, fosfor, vápník a draslík) na složení a produkci rostlinných společenstev, chemické vlastnosti půdy a biomasy, společenstvo mykorhizních hub, jejich výskytu, abundance a dalších parametrů a jejich vzájemné interakce.

5.1 Chemické vlastnosti půdy byly v pokusu Steinach Grassland Experiment průkazně ovlivněny dlouhodobých hnojením, především celkový obsah dusíku, dostupný obsah fosforu, draslíku, vápníku, pH a organické látky v půdě. Bylo zjištěno, že chemické vlastnosti půdy způsobily signifikantní rozdíly v produkci nadzemní biomasy mezi jednotlivými variantami vypočítanou pomocí stlačené výšky porostu. Ačkoliv dlouhodobé hnojení této aluviální louky významně ovlivnilo druhové složení rostlin, nebyly tyto změny natolik významné, jako tomu bylo u ostatních dlouhodobých pokusů. Přežití druhů i v nehnojených kontrolních plochách umožnila především vysoká dostupnost živin a dobrá adaptace rostlin na tyto podmínky. Dospěli jsme tedy k názoru, že rostlinné složení na aluviálních loukách nereaguje na dlouhodobé hnojení dusíkem, fosforem a draslíkem tak dramaticky jako na méně produktivních travních ekosystémech. Dále dlouhodobé hnojení snížilo druhovou rozmanitost rostlin, což bylo dáno přímo půdním okyselováním a nepřímo zvyšováním produkce nadzemní biomasy, která zároveň zvýšila konkurenci o světlo a tím podpořila vyloučení méně konkurenceschopných druhů. Intenzita, s jakou dlouhodobé hnojení dusíkem negativně ovlivnilo druhovou rozmanitost rostlin, záležela na tom, zda byl dusík aplikován samostatně či v kombinaci s dalšími živinami, nebo zda toto hnojení dusíkem způsobovalo okyselování půdy.

5.2 Z výsledků práce vyplynulo, že po sedmdesáti letech hnojení v pokusu Rengen Grassland Experiment byly patrné velké změny v chemických vlastnostech půdy v

jednotlivých variantách obhospodařovaných různým způsobem, především dostupný fosfor v půdě se lišil o celý řád mezi variantami hnojenými a nehnojenými fosforem. Vápnění spolu s aplikací dusíku zvýšilo produkci nadzemní biomasy a podpořilo rozvoj hyf arbuskulárních mykorhizních hub v půdě, zatímco snížilo relativní alokaci uhlíku do kořenů. Současné obohacování půdy o vápník, dusík a fosfor dále zvýšilo produkci nadzemní biomasy, podpořilo trávy a potlačilo ostatní rostlinné funkční skupiny (byliny a leguminózy). Hnojení dusíkem a vápníkem také způsobilo snížení obsahu organického uhlíku v půdě a silně potlačilo arbuskulární mykorhizní houby, ačkoliv reakce na fosfor se u jednotlivých taxonů arbuskulárních hub značně lišily. Zjištěné výsledky indikují, že způsoby zemědělského obhospodařování zaměřené na maximální produkci mohou v dlouhodobém měřítku způsobit změny, které snižují zásoby uhlíku v půdě a mohou tak částečně nebo zcela zamezit potenciálnímu ukládání uhlíky do travních ekosystémů, které slouží jako antropogenního uložiště oxidu uhelnatého.

5.3 Na základě toho experimentu bylo zjištěno, že dlouhodobé hnojení dusíkem, fosforem a vápníkem způsobilo signifikantní změny v chemických vlastnostech půdy, které měly za následek rozdílné růstové odpovědi rostlin. Několik desetiletí trvající hnojení vyvolalo rozpětí půdního pH, které dosahovalo hodnot od slabě kyselého po neutrální. Každoroční aplikace dusíku nijak neovlivnila celkový obsah dusíku v půdě stejně tak jako se vápnění neprojevilo v dostupném obsahu vápníku v půdě. Zcela opačný vliv mělo dlouhodobé hnojení fosforem, které ovlivnilo dostupnou koncentraci fosforu v půdě. Byly pozorovány průkazné rozdíly v růstových odpovědích rostlin pěstovaných v různě hnojených půdách (výška rostlin, počet živých a mrtvých listů a suchá nadzemní biomasa). Nejvyšších hodnot bylo překvapivě dosaženo ve vápněné variantě s přídavkem dusíku, ačkoliv bychom je očekávali v plně hnojené variantě s aplikací vápníku, dusíku a fosforu. Po pěti týdnech trvání pokusu některé rostliny vykvetly, především v kontrolních půdách bez jakéhokoliv přídavku živin, avšak neprůkazně v závislosti na variantě. Z výsledků našeho pokusu vyplývá, že způsob obhospodařování půdy spojený s vyššími dávkami dodaných živin a tím také zvýšenou úrodností půdy může závažně ovlivnit dostupnost a koncentraci živin v půdě, výšku rostlin, suchou nadzemní biomasu a počet listů na rostlině.

CHAPTER 7

CURRICULUM VITAE

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2000 – 2004	Gymnasium Staré Město, Study programme: Ecology and nature protection (high school)

EMPLOYMENT:

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IGA 20124246	Vliv dlouhodobého hnojení na arbuskulární mykorhizní houby oddělení Glomeromycota (2014) - investigator
IGA 20134275	Změny mykorhizních společenstev v důsledku dlouhodobého hnojení (2013) - investigator
IGA20144219	Živinový kontext závislosti trav s C3 a C4 fotosyntézou na mykorrhizní symbióze (2012) - investigator
GAP504/12/1665	Předivo mykorrhizních vláken v půdě: skrytá polovina podzemní ekologie rostlin (2012-2016) - participant
GAČR 14-19191S	Energetické obchody v symbióze – přerozdělování uhlíku v mykorrhizních společenstvech (2014 – 2016) - participant

PROFESSIONAL EXPERIENCE, CONFERENCES AND TRAINING COURSES:

2014	International Congress on Mycorrhizae, Marrakech, Morocco.
2014	Biodiversity 2014, Conference of Department of Ecology, Mělník – Chloumek.
2013	32nd New Phytologist Symposium, Argentina.
2013	Biodiversity 2013, Conference of Department of Ecology, Mělník – Chloumek.
2012	Meeting of PhD students in Plant Ecology and Botany 2012, Podhradí u Ledče nad Sázavou.

2012 Conference: Kostelecké inspirování 2012, Kostelec nad Černými lesy.

2012 Participation on summer school "Scandinavia – Ecosystems of Northern Europe".

2012 Biodiversity 2012, Conference of Department of Ecology, Mělník – Chloumek.

2011 Fifth Meeting of Czech, Slovak and Hungarian PhD students in Plant Ecology and Botany, Piesočná, Slovakia.

2011 Scientific writing course under the auspecies of European Grassland Society

2010 Meeting of PhD students in Plant Ecology and Botany 2012, Podhradí u Ledče nad Sázavou.

PUBLICATION ACTIVITY:

Papers in scientific journals with impact factor:

Hejcman M., **Sochorová L.**, Pavlů V., Štrobach J., Diepolder M., Schellberg J. (2014): The Steinach Grassland Experiment: Soil chemical properties, sward height and plant species composition in three cut alluvial meadow after decades-long fertilizer application. Agriculture, Ecosystems and Environment (184): 76 – 87.

Sochorová L., Jansa J., Verbruggen E., Hejcman M., Schellberg J., Kiers E.T., Johnson N.C. (2016): Long-term agricultural management maximizing hay production can significantly reduce belowground C storage. Agriculture, Ecosystems and Environment (220): 104 – 114.

Gryndler M., Beskid O., Hršelová H., Bukovská P., Hujslová M., Gryndlerová H., Konvalinková T., Schnepf A., **Sochorová L.**, Jansa J. (2015): Mutabilis in mutabili: Spatiotemporal dynamics of a truffle colony in soil. Soil Biology & Biochemistry (90): 62 – 70.

Zemková L. (2016): *Zea mays* responds to 70 years of differently managed soils. Scientia Agriculturae Bohemica (Submitted paper).

Gryndler M., Beskid O., Hujslová M., Konvalinková T., Bukovská P., **Sochorová L.**, Hršelová H., Jansa J. (2016): Soil receptivity for ectomycorrhizal fungi: *Tuber aestivum* is specifically stimulated by calcium carbonate and certain organic compounds. Mycorrhiza (Submitted paper).

Papers in other scientific journals:

Sochorová L., Verbruggen E., Hejcman M., Schellberg J., Jansa J., Johnson N.C. (2013): Arbuscular mycorrhizal community responses to 70-year soil fertilization. 32nd New Phytologist Symposium. Argentina. P95 – 96.

Sochorová L., Jansa J.,Verbruggen E., Hejcman M., Schellberg J., Johnson N.C. (2014): Long-term phosphorus inputs change structure of Arbuscular mycorrhizal fungi. International Congress on Mycorrhizae. Marrakesh, Morocco. P62.

Papers from scientific conferences in Czech:

Sochorová L., Verbruggen E., Jansa J., Johnson N.C., Hejcman M., Schellberg J. (2012): Vliv dlouhodobého hnojení P, Ca a N na kořeny a mykorhizu – Rengen Grassland Experiment. Sborník abstraktů z konference Kostelecké inspirování, FŽP ČZU Praha.

Sochorová L., Hejcman M., Pavlů V., Diepolder M. (2012): Plant species composition and soil chemical properties in alluvial meadow after 75 years of fertilizer application. Sborník abstraktů z konference Biodiverzita 2012, FŽP ČZU Praha.

Sochorová L., Verbruggen E., Hejcman M., Schellberg J., Jansa J., Johnson N.C. (2013): Arbuscular mycorrhizal community responses to 70-year soil fertilization. Sborník abstraktů z konference Kostelecké inspirování, FŽP ČZU Praha.

Sochorová L., Verbruggen E., Jansa J., Johnson N.C., Hejcman M., Schellberg J. (2013): The effect of long-term fertilization on roots and arbuscular mycorrhizal symbioses. Sborník abstraktů z konference Biodiverzita 2013, FŽP ČZU Praha.

Sochorová L., Jansa J., Verbruggen E. (2014): Arbuscular mycorrhizal community responses to 70 years of long term soil management. Sborník abstraktů z konference Biodiverzita 2014, FŽP ČZU Praha.