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Faculty of Science
Department of Ecosystem Biology

Ph.D. Thesis

The impact of outdoor cattle overwintering husbandry on soil fungal community

Mgr. Jiří Jirout

Supervisor: RNDr. Dana Elhottová, Ph.D.
Biology Centre AS CR, v. v. i., Institute of Soil Biology

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Annotation:

The impact of outdoor cattle overwintering husbandry on soil fungal community at upland pastures was investigated. Soils with different intensity of cattle impact were surveyed and the fungal communities were studied by combination of culture-dependent and culture-independent microbiological methods. These analyses showed the substantial shift in biomass, functions, and species composition of fungal communities caused by cattle overwintering.

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Preface and author's contribution

This Ph.D. Thesis is based upon following 3 publications and 3 submitted manuscripts:

I. **Jirout, J.,** Tříska J., Růžičková, K., Vrchotová, N., Šimek, M., Elhottová, D. (*in press*). The comparison of chromatographic methods for ergosterol determination and their application for ergosterol analysis of upland pasture soil [article in Czech]. Chemické listy (IF = 0.593).
Jiří Jirout was responsible for soil sampling, sample preparations, and data assembling, including preparation of the manuscript of the paper.

II. Ascher, J., Ceccherini, M.T., Chroňáková, A., **Jirout, J.,** Borgogni, F., Elhottová, D., Šimek, M., Pietramellara, G. 2010. Evaluation of the denaturing gradient gel electrophoresis (DGGE) - apparatus as a parameter influencing soil microbial community fingerprinting. World Journal of Microbiology and Biotechnology (DOI: 10.1007/s11274-010-0349-z) (IF = 0.945).
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Jiří Jirout participated on preparation of pyrolysis samples, multivariate analyses of data and revision of the manuscript.

IV. **Jirout, J.,** Šimek, M., Elhottová, D. (*submitted manuscript*). The response of soil fungal communities to the disturbing impact of overwintering beef cattle. Soil Biology and Biochemistry (IF = 2.926).
Jiří Jirout was responsible for soil sampling, sample preparations, DNA and fungi isolation, and assembling of data including preparation of the manuscript.

V. **Jirout, J.,** Šimek, M., Elhottová, D. (*submitted manuscript*). The fungal contributors to the emissions of nitrous oxide (N_2O) from cattle overwintering area. Climatic Change (IF = 3.202).
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VI. **Jirout, J.,** Tříska, J., Růžičková, K., Elhottová, D. 2009. Disturbing impact of outdoor cattle husbandry on community of arbuscular mycorrhizal fungi in upland pasture soil. Communications in Soil Science and Plant Analysis 40: 736–745 (IF = 0.462).
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Co-author's agreement

We hereby declare that Jiří Jirout had a major contribution of 1st, 4th, 5th, and 6th paper from the list of papers. His contribution to other papers listed, was also significant.

RNDr. Dana Elhottová, Dr.

Dana

Dr. Judith Ascher

Judith Ascher

prof. Ing. Miloslav Šimek, CSc.

M. Šimek

Maria Teresa Ceccherini, Ph.D.

Maria Teresa Ceccherini

doc. Ing. Jan Tříska, CSc.

prof. Giacomo Pietramellara

RNDr. Naděžda Vrchotová, CSc.

Dr. Federica Borgogni

Federica Borgogni

RNDr. Alica Chroňáková, Ph.D.

Chroňáková Alica

prof. Dr. Michael Schloter

Mgr. Anna Koubová

Koubová

Dr. Andreas Gattinger

RNDr. Tomáš Cajthaml, Ph.D.

Dr. Jürgen Esperschütz

Ph.D. Thesis – content:

CHAPTER 1

GENERAL INTRODUCTION AND AIMS

1 GENERAL INTRODUCTION

1.1 Temperate pastures and land use changes

Upland and mountainous agricultural areas worldwide have been usually used as pastures for cattle or sheep (Kohler et al., 2005). Using Food and Agriculture Organization's (FAO) data and definition, the world area of pasture was estimated to be 35 millions km^2 in year 2000, representing 26 % of the world land area and 70 % of the world agricultural area (FAO, 2008). Although temperate pasture ecosystems cover approximately 10 % of the land area in Europe (Soussana et al., 2004), microbial communities in pasture soils have been under less attention then in arable and forest soils (Iyyemperumal et al., 2007).

The intensive human activities and land use changes during last century triggered dramatic shift in the function and diversity of all terrestrial ecosystems (Sala et al., 2000), including upland pastures. Efficient and sustainable management of currently used pasture soils requires knowledge about all organisms involved in these ecosystems (McCracken and Bignal, 1998) equally as knowledge about the effects of cattle on their communities. Soil microorganisms play a crucial role in grassland ecosystems (Clegg, 2006). Changes in their communities affect nutrient cycling (Clegg, 2006), quality, stability, and fertility (Potthoff et al., 2006; Hijri et al., 2006), as well as productivity and biodiversity of upland grasslands (Bardgett, 1996).

1.2 Outdoor cattle overwintering husbandry and its impact on soil environment

Agriculture practices have been recently focused on the development of sustainable system of cattle husbandry (Redbo et al., 1996). Outdoor cattle husbandry is widespread and sustainable way of use of upland grasslands. Moreover, the rising housing costs led to the demand for inexpensive winter housing systems, such as outdoor wintering (overwintering) of cattle (Manninen et al., 2008). Nowadays, specific parts of pastures are used as overwintering areas, typically from October to May in Western and Central Europe (Wassmuth et al., 1999; Šimek et al., 2006). The fundamental changes in the pasture environment are caused by the gathering of animals near open barns (Wassmuth et al., 1999). The cattle activities are considered as major biotic factor affecting the structure and dynamics of pasture ecosystems (Bokdam & Gleichman, 2000). The effect of outdoor cattle overwintering husbandry on pasture environment consists of 1) vegetation removals (Wassmuth et al., 1999), 2) excretal returns connected with greenhouse gases (GHG) emissions (Šimek et al., 2006), and 3) trampling down of the soil surface and compaction of deeper soil layers (Kohler et al., 2005).

The majority of nutrients (60 - 95 %) from cattle plant diet returns into the soil in a form of dung and urine (Haynes & Williams, 1999). The excrements of beef cattle are distributed mainly randomly (Shand et al., 2000). Places with higher excrements accumulation are connected mainly with resting or sleeping places (White et al., 2001; Kohler et al., 2006). Concentrations of total nitrogen (N_{tot}), organic carbon (C_{org}), and available phosphorus (P) as well as pHs are increased in cattle impacted soils (Šimek et al., 2006). The high amount of N and P from released excrements initializes dramatic changes of structure and diversity of all soil organisms (Frey et al., 2004). The mechanical degradation caused by the cattle trampling leads into the creation of patches without vegetation which are susceptible for more severe water and wind erosion.

1.3 Changes in microbial communities under outdoor cattle overwintering husbandry

The outdoor cattle overwintering husbandry causes dramatic changes in the soil microbial community biomass and structure (Williams et al., 2000). Excretal returns into soil trigger substantial changes of communities of archaea (Radl et al., 2007) and bacteria (Philippot et al., 2009), especially bacterial denitrifiers (Chroňáková et al., 2009), which reduce nitrates or nitrites in soil to nitrogen oxides or even dinitrogen. The higher abundance of denitrifiers and archaea in the impacted soils of the overwintering area leads into the increased emissions of GHG (methane and nitrous oxide) (Šimek et al., 2006; Hynšt et al., 2007; Brůček et al., 2009).

Right after excrements deposition on the soil surface, the activity, biomass, and species composition of microbial community are affected (Hernández et al., 2007; Plaza et al., 2007). The intensive nutrients inputs from excrements caused suppression of fungal biomass (de Vries et al., 2006), particularly the arbuscular mycorrhizal fungi reduction can be expected. On the other hand, dramatic short-term increment was observed in the biomass of proteolytic, amonifying, and nitrifying bacteria (Acea and Carballas, 1988; Williams et al., 2000; Petersen et al., 2004). Based on the phospholipid fatty acid (PLFA) analyses after deposition of artificial urine, Petersen et al. (2004) observed the enrichment of soil by branched fatty acids i14:0, a15:0 and i17:0, markers for Gram-positive bacteria (Zelles, 1997). Nevertheless, the high microbial activity at nutrient hot-spots lead into the increase of anteiso- to iso-branched fatty acids ratio, which is characteristic for the developing of anaerobic conditions and the increase of anaerobic bacterial biomass there (Frostegård et al., 1997). These conditions are favoured by the denitrifiers and anaerobic nitrogen fixing bacteria. On the other hand, the lack of mid-branched fatty acids typical for actinomycetes (10Me17:0, 10Me18:0) (Frostegård et al., 1997) and reduction of 18:2ω6 fatty acid typical for fungi (Federle, 1986) implies that the nitrogen inputs suppress the actinomycetal (Nunan et al., 2006) as well as fungal community (Petersen et al., 2004).

1.4 Soil fungi and their role in pasture ecosystems

Soil fungi, which could embody up to 90 % of microbial biomass in temperate soils (Kjøller & Struwe, 1982), perform in pasture ecosystems essential services for the water dynamics, nutrient cycling, vegetation development and disease suppression (Deacon et al., 2006; Ingham, 1999). The Ph.D. thesis was focused on two most important groups of fungi in soil – arbuscular mycorrhizal fungi (*mutualists*) and saprotrophs (*decomposers*). *Mutualistic* mycorrhizal relationships between plants and mycorrhizal fungi belong to the most important interaction for plants survival at pastures (Hunt et al., 2004). Mycorrhizal fungi colonize plant roots, help to protect plants from pathogens, help to solubilise phosphorus and bring soil nutrients to the plant in exchange for carbon from the plant (Ingham, 1999). Fungi in pastures play also important role as *decomposers* (saprotrophs) in the soil. Using diverse enzymes (Baldrian, 2009; Baldrian et al., 2010), fungi convert hard-to-digest organic material (aromatic lignin compounds) into more simple molecules accessible for other microorganisms and plants (Ingham, 1999; Bárta et al., 2010). Recently, substantial emissions of GHGs (methane and nitrous oxide) from pasture soils under cattle overwintering husbandry were described (Radl et al., 2007; Hynšt et al., 2007; Brůček et al., 2009). There is also the potential to involve soil fungi into nitrous oxide production in such a nitrogen saturated environment, because this ability was recently pronounced within several common soil fungi (Kurakov et al., 2000).

Despite the above mentioned roles of soil fungi in pasture ecosystems, there is still paucity of data about the fungal communities in soils under cattle outdoor overwintering husbandry. Therefore, this Ph.D. thesis was focused on the aspects of fungal biomass, community structure and specific functions of selected groups of fungi in association with different impact of outdoor cattle overwintering husbandry on pasture soil environment.

2 METHODS TO STUDY FUNGAL COMMUNITY IN PASTURE SOIL

2.1 Quantitative analysis

The information obtained by the combination of traditional (culture-dependent) and modern (culture-independent) methodology could provide the complex insight regarding the response of soil fungal community to the outdoor cattle overwintering husbandry. The use of specific media and conditions for fungal cultivation enable to observe only 5 to 15 % of all fungi (Hawksworth, 1991; Bridge & Spooner, 2001). Modern microbial methodologies based on genotypic or phenotypic techniques can overcome the problems associated with culturing (Clegg, 2006). These approaches can be used for the monitoring and evaluating of changes in complex fungal communities (Leckie, 2005).

For the purpose of the Ph.D. thesis, the quantity of saprotrophic fungi (*decomposers*) in soils was assessed by a set of methods: colony forming units (CFU) and biomarker assays for ergosterol and phospholipid fatty acids (PLFA 18:2ω6) (Frankland et al., 1990). However, all these methods are somehow biased. CFU numbers show all fungal propagules including dormant spores, not only the living mycelium (Smit et al., 1999). Biomarker methods are highly influenced by the various concentrations of biomarker molecules within the tissues of different fungal species (Stahl & Parkin, 1996). Despite known difficulties, use of ergosterol and PLFA 18:2ω6 was widely accepted by scientists as a reliable, robust and relatively inexpensive method for the fungal biomass assay (Montgomery *et al*., 2000; Klamer & Bååth, 2004; Young et al., 2006; Zelles et al., 1992).

The use of ergosterol was not suitable for *mutualistic* arbuscular mycorrhizal fungi in pasture soil - AMF contain negligible amounts of this sterol in their cell walls. The analysis of phospho/neutral lipid fatty acids (PLFA or NLFA 16:1ω5) enabled AMF biomass estimation (Olsson et al., 1998). For the specific determination of AMF content in soil, methods based on extraradical or intraradical mycelium are used. The length of extraradical mycelium after membrane filtering and staining was used by Jakobsen et al. (1992) and Giovanneti & Mosse (1980). Since the mycorrhizal fungi play a crucial role in the mycorrhizal symbiosis with vascular plants, the detection of AMF fungi inside the plant roots remains as the major method for the estimation of AMF content in the environments (Vierheilig et al., 2005). The visualization of AMF structures in roots was possible after staining (Kormanik & McGraw, 1982) and the quantity of AMF structures was determined by magnified gridline intersection method (McGonigle et al., 1990).

2.2 Qualitative analysis

The culture-dependent qualitative methods for saprotrophic fungi were based on the macro- and microscopic observations of morphological features and determination according to the specific keys (Domsch et al., 1980). Also arbuscular mycorrhizal fungi can be identified on the basis on hyphae and spores morphology (Morton, 1988). These methods are, however, seriously biased by the subjectivity of the observer.

To overcome the bias of culture-dependent methods, the genotypic culture-independent methods were used in our project after the extraction and purification of DNA from soil (Miller et al., 1999). The key factor for the use of genotypic methods was the application of polymerase chain reaction (PCR) for the amplification of target DNA regions (Bridge & Spooner, 2001). The amplified 18S rDNA was used in sequencing and fingerprinting analysis (Kowalchuk et al., 1997a). The DGGE method (denaturing gradient gel electrophoresis; Muyzer et al., 1993; Kowalchuk et al., 1997b) was very useful fingerprinting method for the separation of PCR products of environmental samples and allowed comparing multiple samples within one run of the analysis (Agnelli et al., 2004). The phenotypic methods based on lipid fatty acids (PLFA and NLFA) (Leckie, 2005) also provided powerful tool for the creation of phenotypic fingerprints of soils under study (Zelles, 1997).

3 AIMS AND HYPOTHESIS OF THE PH.D. THESIS

The aim of the Ph.D. thesis was to compare the communities of soil microorganisms, especially fungi, in the disturbed and undisturbed parts of cattle overwintering area, and to evaluate the changes of fungal services in terms of biomass, functions, and species composition of fungal communities in pasture ecosystem affected by the outdoor cattle overwintering husbandry. The Ph.D. thesis is based on three years period of *in situ* monitoring and *in vitro* experiments and the use of combination of culture-dependent and culture-independent methods of fungal community profiling. Our main hypothesis was that the outdoor cattle overwintering husbandry at the upland pasture negatively affects the fungal biomass as well as species richness and composition of fungal communities.

The particular hypotheses of the Ph.D. were as follows:

1) The soil disturbances caused by cattle have significant negative effect on quantity and quality of fungal community.
2) The diversity of fungal community decrease along the cattle impact intensity.
3) Selected functional groups of soil fungi are affected with respect to their role in soil ecosystem.

CHAPTER 2

SITE DESCRIPTION AND SAMPLINGS

The presented hypotheses were proved at the specific pasture ecosystem, where unique gradient of cattle impact on soil environment has developed (Fig. 1). The experimental area was located at an upland pasture used as an overwintering area for beef cattle at Borová, 10 km northwest from Český Krumlov, South Bohemia, Czech Republic (GPS: 48°52´N, 14°13´E) in the Landscape Protected Area Blanský les. The mean annual temperature at the area is 7°C and annual sum of rainfall is 650 mm. The soil type was classified as Haplic Phaeozem (arenic, WRB system) with 60-80% of sand, 14-26% of silt, and 6-14% of loam (USDA classification system) (Chroňáková et al., 2009). Based on the previous experiments conducted on the same overwintering pasture (Šimek et al., 2006; Hynšt et al., 2007; Radl et al., 2007; Chroňáková et al., 2009), the area could be separated into three main parts. Two locations represents soil with different intensity of disturbances (S - site with strong cattle impact, and M - site with moderate impact); 1 location represents undisturbed soil (C - control with no impact) (Šimek et al., 2006). The surrounding area was formerly used as permanent grassland for hay production. Therefore, the perennial vegetation comprises of the mix of graminoids, legumes and other vascular plants. However, the locality has been used as an extensive pasture for outdoor cattle husbandry since 1995. Approximately 90 cows use the open barn connected with 4.04 ha of overwintering area usually from October till May each year.

The soil samples for consequent analyses were collected during three years of experimental period from S, M, and C locations directly after cattle abandoned the overwintering area (usually beginning of May) and right before the gathering of cattle for overwintering (usually beginning of October). Three independent samples from each location consisted of three subsamples thoroughly sieved and mixed. The average chemical parameters of soils from S, M and C parts of the overwintering pasture from 3 years of the experimental lasting are summarized in Table I.

Table I. Average values of selected chemical parameters of upland pasture soil under different intensity of cattle impact during three years of the experimental lasting (mean ± SD; n = 9). The letters (a-c) indicate significant differences between sampling locations. The significance level was at $p \leq 0.05$.

		S	**M**	**C**
pH	in $CaCl_2$	7.38 ± 0.28a	6.48 ± 0.21b	4.96 ± 0.12c
Corg	%	14.61 ± 2.74a	9.08 ± 1.02b	5.96 ± 0.85c
Ntot	%	0.67 ± 0.04a	0.37 ± 0.09b	0.18 ± 0.02c
C:N		23.67 ± 3.09a	26.19 ± 4.89a	32.68 ± 4.31a
P	mg P g^{-1} dw	0.64 ± 0.09a	0.21 ± 0.02b	0.01 ± 0.00c
K	mg K g^{-1} dw	3.00 ± 0.84a	1.51 ± 0.40b	0.07 ± 0.06c
Mg	mg Mg g^{-1} dw	0.83 ± 0.09a	0.37 ± 0.03b	0.18 ± 0.02c
Ca	mg Ca g^{-1} dw	4.53 ± 0.08a	2.13 ± 0.33b	1.31 ± 0.04c

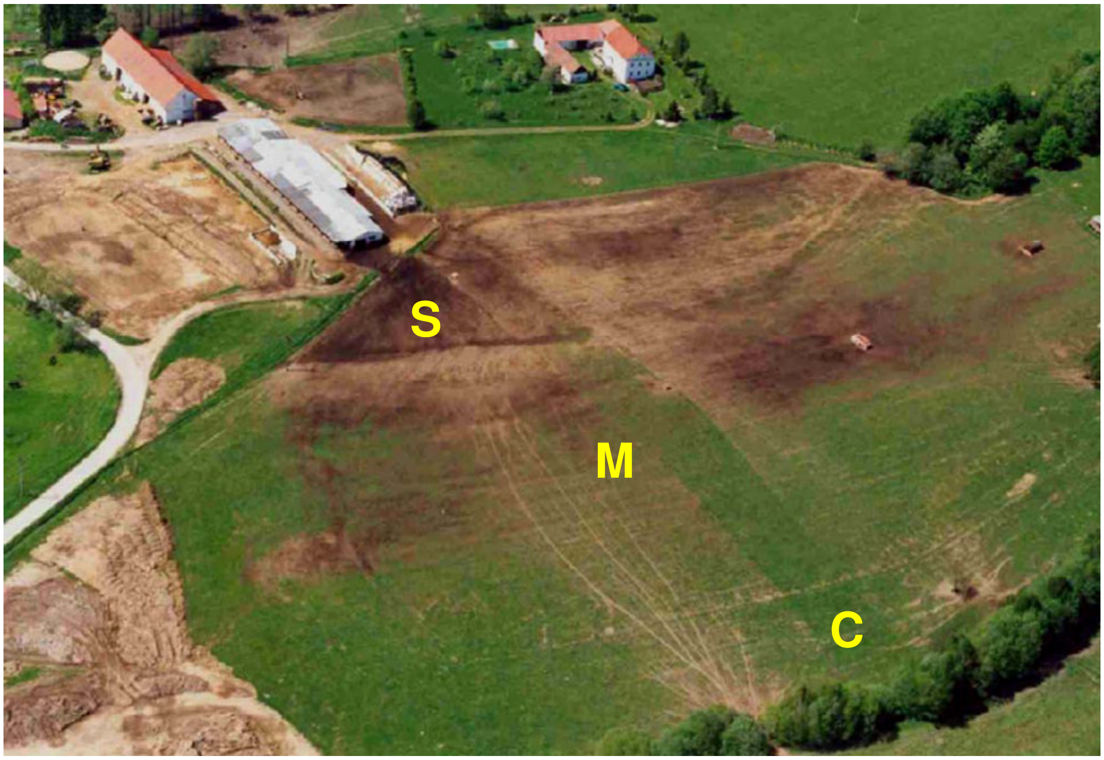

Fig. 1. The aerial view on the study overwintering area with the three different study sites (S, M, and C) along to the gradient of cattle disturbing impact (S - severe impact, M - medium impact, C - no impact). Beef cattle farm Borová, Czech Republic (48°52´ N, 14°13´ E). Photo by M. Šimek.

CHAPTER 3

RESULTS AND GENERAL DISCUSSION

The first part of the dissertation deals with methods which were used for the analyses of studied fungal communities and potentially improved and optimised for the use in general microbial ecology. The eligibility of the three most often used chromatographic techniques was investigated with connection to the ergosterol determination in the ecosystem under study (**Paper I**). The selective MS-MS mode of GC-MS technique represents more advantageous approach for determination of ergosterol in the complex matrices such as soil than the most often used HPLC technique. The GC-MS-MS eliminates possibly coeluting peaks of similar compounds (Verma et al., 2002) or interferences with environmental matrices (Abramson and Smith, 2003; Headley et al., 2002) occurring during HPLC analyses. The results of conducted experiments showed the significant increase in fungal biomass along the increasing cattle impact from 0.93 (C soil) to 29.28 μg of ergosterol per gram of S soil. GC-MS in the full scan mode represented powerful method for monitoring of complex sterols and their transformations in the environmental samples. Full scan GC-MS revealed enrichment of cattle impacted soils by non-fungal sterols (campesterol, stigmasterol and β-sitosterol) and stanols (ergostanol, sitostanol). They originated in digested plant material and rumen microflora (Rogge et al., 2006). These results suggested significant changes in soil biochemistry of the pasture soils under outdoor cattle overwintering husbandry.

The methodological aspect of the use of the two most often DGGE systems (BioRad vs. Ingeny) as a parameter impacting the assessment of microbial community structures was investigated in **Paper II**. The DGGE conditions were chosen as similar as possible, to avoid other variables than the apparatus itself. The comparison of individual obtained results indicated that the DGGE apparatus itself is the factor influencing DGGE analysis. The DGGE systems introduce inter-gel variability, due to different technical characteristics of the DGGE systems themselves (gradient former, heating device, dimensions of the gel, buffer circulation, etc.) (Green, 2005). From these reasons one and the same system throughout an experiment was used, if multiple gel-to-gel analyses were required.

The second part of the dissertation summarises results of the three years monitoring period, which demonstrated the impact of cattle overwintering activities on both whole soil microbial and soil fungal community (**Paper III – VI**). The results of all performed analyses proved that the disturbances of soil environment at the cattle overwintering area caused by intensive trampling and inputs of cattle excrements to the soil lead to the changes in the soil microbial communities. Total microbial biomass (**Paper III**) as well as biomass of soil fungi (**Paper IV**) at the sites affected by overwintering cattle increased as a result of stimulation by increasing level of both labile and stable dung C, which is incorporated to the soil by cattle trampling during winter season. Results of the **Paper IV** were surprising, because they are in the contrast with the other published data about cattle suppressing effect on fungal biomass and species richness (de Vries et al., 2006), fungal acidic pH optima (Brady and Weil, 2001) and susceptibility of filamentous hyphae of soil fungi to mechanical disturbation (Klein and Paschke, 2000). The stimulating effect of intensive inputs of organic carbon and total nitrogen from cattle excrements on the fungal biomass and diversity exceeded the negative aspects of cattle overwintering activities.

The relationship between soil organic matter (SOM) and microorganisms was elucidated by comparison of the profiles of organic matter pyrolytic products (OMPP) (**Paper III**). OMPP of S soil and excrements contained mainly recalcitrant lignin structures (H compounds) originated in plants (Martínez et al., 2005). The decreased levels of lignin compounds in M and C soils could be explained by its degradation by basidiomycetous (myccorhizal) and ascomycetous fungi (Hatakka, 2001). On the other hand, OMPP of the M soil were enriched by fragments of polysaccharides or polymaleic acid (L compounds) (Saiz-Jimenez, 1994), which are usually linked with the fresh SOM (Vancampenhaut et al., 2009) and accumulate under conditions favourable for decomposition processes (Grandy et al., 2009). The discrepancy in SOM composition of M soil in spring and autumn indicated substantial decomposition processes of SOM accumulated during winter. The loss of H and L compounds as well as increase of compounds containing N and P is linked with the high degree of SOM decomposition, plant roots development and high microbial activity as reported by Schulten et al. (1992).

The sequencing analyses of fungal community (**Paper IV**) revealed several ecological and/or physiological groups of the fungi: plant pathogens (*Alternaria* sp., *Fusarium* sp.), saprotrophic fungi (*Penicillium* sp., *Rhizopus* sp., *Trichoderma* sp.), insect pathogens (*Isaria* sp., *Metarhizium* sp.), and coprophilous fungi (*Zygorhynchus* sp., *Pilobolus kleinii*). The species composition is highly similar to those previously published for soils in temperate region – several species of *Aspergillus*, *Mucor* and *Penicillium*, together with *Trichoderma*, C*ladosporium*, *Alternaria*, *Rhizopus*, and *Fusarium* (Pugh, 1974).

The significant decrease of fungal biomass in S soil in spring contrary to autumn could be explained by developing of anaerobic conditions in soil after cattle excretions (Bossio and Scow, 1998) or by toxicity of ammonium to soil microorganisms (Müller et al., 2006), including fungi (Hess et al., 2006). The decrease of fungal biomass in C soil in spring contrary to autumn was affected by growing plants, which started to use available P immediately after the vegetation season started and caused phosphorus depletion (Schachtman et al., 1998). The cultivable part of fungal community also could be influenced by the seasonality of individual fungal species occurrence (de Ana et al., 2006).

The higher diversity of microorganisms in cattle impacted S soil is connected with cyclic inoculation of soil by many of the new microorganisms originated in cattle excrements (**Paper III and IV**). Polar lipids analysis (indicator PLEL ip20:1 for archaea, NEL-PUFA for anaerobic fungi, and NEL-br-STFA for anaerobic Gram-negative bacteria) (Zelles, 1997; 1999) confirmed new microbial community profile in the most impacted soil derived from a cattle intestine microflora. The S soil contained high amounts of archaeal lipids, resulting from high and repeated inputs of cattle manure (**Paper III**). As it was described by Gattinger et al. (2007), high organic fertilization increases archaeal biomass, which could be mainly related to methanogenic *Methanoculleus* and *Methanosarcina* species (Radl et al. 2007). The biomass of anaerobic Gram-negative bacteria was also increased in the most impacted S soil. The presence of anaerobic fungi in fresh cattle excrement and in the most disturbed soil (S) was proved by DNA sequencing (**Paper IV**). This fungal group belongs to the phylum Neocallimastigomycota, which contains anaerobic, cellulose degrading rumen fungi (Orpin, 1975; Bauchop, 1979).

The presence of anaerobes in S soil is possible due to anaerobic microhabitats (Radl et al., 2007), resuled from dramatic changes of the soil surface during non-vegetation period each year. The upper layer (20 cm) of soil profile is repeatedly flooded with cattle urine, saturated with cowpats (Šimek et al., 2006) and high microbial activity could cause oxygen depletion (Frostegård et al., 1997). Especially in spring, soil is saturated by water from melting snow and the increased temperature as well as accumulated nutrients from excrements boosts microbial activity, resulting in emissions of greenhouse gases. **Paper V** showed the involvement of soil fungi in emissions of nitrous oxide (N_2O), a very potent greenhouse gas, from soil to the atmosphere.

The potential of N_2O production by fungi was suggested in places with high inputs of nitrogen and often presence of anaerobic microhabitats. The results presented in **Paper V** confirmed this presumption and showed that a wide range of common soil fungi isolated from cattle overwintering area have the ability to produce large amounts of N_2O in laboratory conditions. Therefore, further research is needed to elucidate the significance of fungal N_2O production in the field. Several fungi are capable to use nitrite molecules as an energy source during denitrification in the conditions of lower partial O_2 pressure (Watsuji et al., 2003; Morozkina and Kurakov, 2007), but they are also capable to detoxify NO from their cells (Morozkina and Kurakov, 2007) or utilize different nitrogen substrates in a process of codenitrification (Tanimoto et al., 1992, Laughlin and Stevens, 2002), with nitrous oxide as major product of these fungal driven nitrogen tranformations.

The substantial negative effect of outdoor cattle overwintering husbandry was observed in the case of arbuscular mycorrhizal fungi (**Paper VI**). The intensity of mycorrhizal symbiosis, revealed as the amount of mycorrhizal structures inside the host plant roots and the length of extraradical hyphae, decreases along the increasing cattle impact at the overwintering area. The negative effect of increased concentration of N_{tot} and available P were in accordance to findings of Egerton-Warburton et al. (2001) and Kahiluoto et al. (2001), who described the suppression of AMF in soil and host plant roots after N and P fertilization. The plant community was also altered by the cattle overwintering husbandry. The control site was represented by the typical vegetation of upland meadow, containing several species of grasses and forbs usually forming an extensive mycorrhizal symbiosis (Sanders et al., 1996), whereas at the impacted sites (S and M) the meadow species were replaced by rural species which do not form mycorrhizae (Wang and Qiu, 2006).

CONCLUSION

Generally, the results of the Ph.D. thesis demonstrated that the cattle overwintering activities (trampling and deposition of excrements on the soil surface) triggered substantial changes in soil chemical conditions, as well as the increase in biomass and diversity of soil microbial communities, including soil fungi. The hypothesised negative aspects of cattle overwintering activities on the fungal biomass and diversity were exceeded by stimulating effect of intensive inputs of organic carbon and total nitrogen from cattle excrements. Outdoor cattle overwintering husbandry also caused the significant shifts in the species composition and introduction of new species of fungi at the impacted parts of upland pastures for cattle overwintering, where the enrichment of soil by organic matter favours anaerobic conditions and GHG production. The information obtained by the combination of culture-dependent and -independent methodology provides the complex insight regarding the composition and response of soil fungal community to the different intensity of cattle impact. The three functional fungal groups showed different occurrence pattern: promotion of fungal decomposers and N_2O producers at the expense of reduction of arbuscular mycorrhizal fungi. Contrary the role of fungi in upland pastures (incorporation of nutrients into fungal biomass), soil fungi at the overwintering area contributed to the losses of nutrients from this ecosystem.

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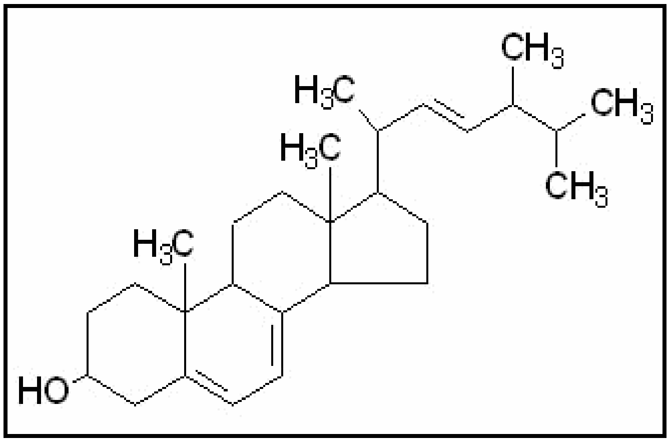
CHAPTER 4

RESEARCH ARTICLES

I



Jirout, J., Tříska J., Růžičková, K., Vrchotová, N., Šimek, M., Elhottová, D. (*in press*). The comparison of chromatographic methods for ergosterol determination and their application for ergosterol analysis of upland pasture soil [article in Czech]. *Chemické listy.*

Jiří Jirout was responsible for soil sampling, sample preparations, and data assembling, including preparation of the manuscripts of the paper.

Následující pasáž o rozsahu 12 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena pouze v archivovaném originále disertační práce uloženém na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

ČSCH

Chemické listy

Česká společnost chemická
Redakce časopisu Chemické listy
Novotného lávka 5
116 68 Praha 1
tel. 221 082 370, tel., fax 222 220 184
e-mail: chem.listy@csvts.cz

Jiří Jirout
Katedra biologie ekosystémů
PřF Jihočeská univerzita
Branišovská 31
370 05 České Budějovice

V Praze dne 18.2.2010

Vážený pane autore,

tlumočím Vám rozhodnutí redakční rady. Váš rukopis 057/09 s názvem „Porovnání chromatografických metod pro stanovení ergosterolu a jejich využití při analýze půdy podhorské pastviny“ autorů Jiří Jirout, Jan Tříska, Kamila Růžičková, Naděžda Vrchotová, Miloslav Šimek a Dana Elhottová byl přijat k tisku a bude v nejbližším možném termínu opublikován v našem časopise Chemické listy.

S pozdravem za redakci

Radmila Řápková

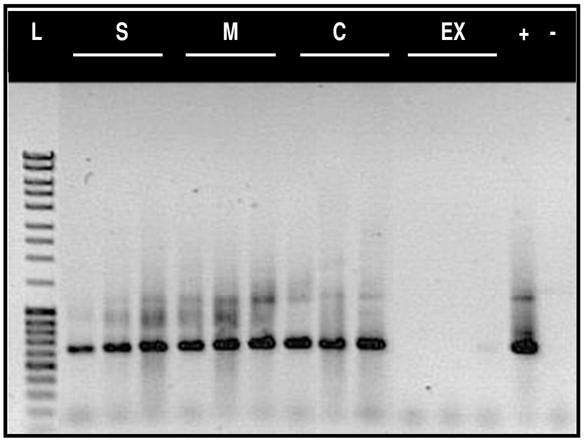
Ing. Radmila Řápková
technická redaktorka

CHEMICKÉ LISTY
REDAKCE
Novotného lávka 5, 116 68 Praha 1
ČESKÁ REPUBLIKA

Abstract: Ergosterol is a biomarker of living saprotrophic fungi. It was extracted from soil by microwave assisted extraction and its concentration in soil was determined using different techniques: GC-MS-MS, HPLC, and GC-FID, respectively. The GC-MS-MS technique was used to monitor fungal biomass in the upland pasture soils differently impacted by overwintering cattle. The amount of ergosterol in soil increased from 0.93 ± 0.34 to 29.28 ± 3.07 $\mu g.g^{-1}$ dw of soil along with increasing cattle impact. The differences between soils were likely caused by relatively high inputs of organic matter in the form of cattle excrements in the soils under study. The limit of detection of both GC-MS-MS and HPLC methods for detection of ergosterol was ca 10 times better in comparison to GC-FID method. Whereas GC-MS in the full scan mode represented the best method for monitoring of complex sterols profile in the environmental samples, consequent selective MS-MS mode allowed determination of ergosterol in the complex matrices by elimination of possibly coeluting peaks. The enrichment of cattle impacted soils by non-fungal sterols (campesterol, stigmasterol and β-sitosterol) and stanols (ergostanol, sitostanol), originated in digested plant material and rumen microflora, suggests significant changes in soil biochemistry of the pasture soils under study.

Abstrakt: Ergosterol je využíván jako biomarker živých saprotrofních hub. Ergosterol byl izolován z půdy mikrovlnnou extrakcí a jeho koncentrace v půdě byla stanovována pomocí různých technik: GC-MS-MS, HPLC a GC-FID. GC-MS-MS metoda byla použita ke sledování biomasy hub v půdách podhorské pastviny s rozdílnou intenzitou zátěže přezimujícím skotem. Množství ergosterolu v půdě rostlo se zvyšující se intenzitou zátěže skotem od 0,93 ± 0,34 do 29,28 ± 3,07 $\mu g.g^{-1}$ suché půdy. Rozdíly mezi studovanými půdami byly nejspíše způsobeny relativně vysokými vstupy organické hmoty z exkrementů skotu do půdy. Detekční limit pro stanovení ergosterolu metodami GC-MS-MS a HPLC byl cca 10krát lepší ve srovnání s metodou GC-FID. Zatímco metody GC-MS v režimu „full scan“ představuje nejlepší metodu pro stanovení komplexního profilu sterolů v přírodních vzorcích, následný selektivní MS-MS režim umožňuje detekci a determinaci ve složitých matricích díky eliminaci látek, které by mohly koeluovat s hledaným analytem. Obohacení půd ovlivněných skotem o steroly (campestrerol, stigmasterol, β-sitosterol) a stanoly (ergostanol, sitostanol), pocházející z nestráveného rostlinného materiálu a bachorové mikroflóry naznačuje významné změny v biochemii studovaných půd.

II



Ascher, J., Ceccherini, M.T., Chroňáková, A., **Jirout, J.,** Borgogni, F., Elhottová, D., Šimek, M., Pietramellara, G. 2010. Evaluation of the denaturing gradient gel electrophoresis (DGGE) - apparatus as a parameter influencing soil microbial community fingerprinting. World Journal of Microbiology and Biotechnology (DOI: 10.1007/s11274-010-0349-z).

Jiří Jirout participated on the soil sampling, results evaluation, and revision of the articles.

Následující pasáž o rozsahu 6 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena v archivovaném originále disertační práce uloženém na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Abstract: We compared two denaturing gradient gel electrophoresis (DGGE) systems – Dcode (Biorad, Hercules, CA, USA) and PhorU (Ingeny, Leiden, NL), performing community level 16S and 18S rRNA gene fragment-PCR-DGGE with total DNA extracted from upland pasture soil used for outdoor cattle husbandry. The methodological evaluation of the DGGE apparatus as parameter influencing DGGE fingerprinting, based on cluster analysis of soil bacterial and fungal community fingerprints, was made in terms of the resulting information about microbial community structures and their response to different degrees of cattle impact. Although the comparative DGGE analysis with different DGGE systems provided similar clustering of microbial community structures in correlation with the degree of cattle impact, our results suggest the DGGE system to be a factor influencing DGGE analysis. To our knowledge this is the first attempt to investigate the hypothetical impact of the DGGE system due to different technical characteristics, recommending the use of one and the same DGGE apparatus throughout an experiment, if the monitoring of microbial community structures requires multiple gel-to-gel analysis.

Abstrakt: Porovnávali jsme dva systémy denaturační gradientové gelové elektroforézy (DGGE) – Dcode (Biorad, Hercules, CA, USA) a PhorU (Ingeny, Leiden, NL) pro analýzy společenstev na základě fragmentů genů pro 16S a 18S rRNA. Celková DNA byla extrahována z půdy podhorské pastviny využívané pro venkovní pastvu skotu a amplifikována pomocí PCR. Zhodnocení DGGE přístroje jako parametru, který ovlivňuje výslednou DGGE analýzu bylo založeno na klusterové analýze fingerprintu půdního hubového a bakteriálního společenstva. Zhodnocení bylo provedeno z hlediska výsledných informací o struktuře mikrobiálního společenstva a jeho odpovědi na rozdílnou intenzitu zátěže skotem. Ačkoliv srovnávací DGGE analýza různých DGGE systémů poskytuje podobné výsledky klastrování profilů mikrobiálních společenstev korelující se intenzitou zátěže skotem, naše výsledky naznačují, že DGGE systém může být dalším faktorem ovlivňujícím DGGE analýzy. Domníváme se, že jde o první pokus o zhodnocení hypotetického vlivu DGGE systémů na základě jejich různých technických znaků, proto doporučujeme používat vždy jen jeden a ten samý DGGE přístroj v případě, kdy je nutné sledovat mikrobiální společenstvo za použití více DGGE gelů.

III

Elhottová, D., Koubová, A., Šimek, M., Cajthaml, T., **Jirout, J.,** Esperschuetz, J., Schloter, M., Gattinger, A. (*submitted manuscript*). Changes in soil microbial communities as affected by intensive cattle husbandry. *Applied Soil Ecology*.

Jiří Jirout participated on preparation of pyrolysis samples, multivariate analyses of data and revision of the manuscript.

Následující pasáž o rozsahu 20 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena v archivovaném originále disertační práce uloženém na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Abstract: Soil microbial communities play central roles in organic matter transformation and hence in soil organic matter (SOM) composition. The present field study documented substantial changes in the microbial community in an upland pasture soil resulting from 10 years of "cattle outdoor over-wintering practice". Extended polar lipids analysis (PLA) confirmed a qualitatively new microbial community profile and a four-fold increase in the size of the microbial community in the heavily impacted soil. The new microbial community was derived from a cattle intestine microflora that had established in the impacted soil. Relative to a nonimpacted soil, the heavily impacted soil contained high and stabile contents of archaea and also high but less stabile contents of bacterial and fungal anaerobes. Increased microbial growth in the heavily impacted soil was confirmed by MUFA/STFA and cyclopropyl-PLFA indicators. The heavily impacted soil also had an increased nutrient content. A significant reduction of the actinobacteria during the winter was the only temporary change in the heavily impacted soil. The significant increase in SOM, which had a high content of aromatic compounds and the same pyrolytic profile as the cattle excrements, was identified as the main factor for the qualitative stability of the new microbial community in the cattle-impacted soil. The study also documented microbial resilience in a moderately impacted soil. During the growing season, the moderately impacted soil lost 75% of the C_{org} that had accumulated during winter; its aromatic-rich-SOM showed significant transformation in SOM, enriched by N, P-organic derivates. Results in this study showed that the stability of the microbial changes due to the cattle outdoor over-wintering practice depended on the stability of the quantitative and qualitative changes of the SOM-impacted soil.

Abstrakt: Půdní mikrobiální společenstva hrají hlavní roli při transformaci organické hmoty a tudíž i při vytváření půdní organické hmoty (POH). Předkládaná terénní studie dokumentuje významné změny ve společenstvu mikroorganismů v půdě podhorské pastviny způsobené 10 lety praxe venkovního přezimování skotu. Rozšířená analýza polárních lipidů (PLA) potvrdila kvalitativně nové mikrobiální společenstvo o čtyřnásobně vyšší biomase v silně ovlivněné půdě ve srovnání s půdou neovlivněnou. Nové mikrobiální společenstvo bylo odvozeno z mikroflóry trávicího traktu skotu, která osídlila silně ovlivněnou půdu. V porovnání s neovlivněnou půdou došlo v silně ovlivněné půdě k stabilnímu zvýšení archaeí a také k zvýšení anaerobních bakterií a hub, které však bylo méně výrazné. Zvýšený mikrobiální růst v silně ovlivněné půdě byl potvrzen MUFA/STFA a cyclopropyl-PLFA indikátory. Silně ovlivněná půda vykazovala také zvýšený obsah živin. Statistický průkazná redukce aktinobakterií během zimního období byla jedinou dočasnou změnou v silně ovlivněné půdě. Významný nárůst POH s vyšším obsahem aromatických sloučenin a podobnost pyrolytického profilu s profilem exkrementů, byly identifikovány jako hlavní faktory pro stabilizaci nového mikrobiálního společenstva v silně zatížené půdě. Studie dále dokumentuje resilienci mikrobiálního společenstva ve středně zatížené půdě. Během vegetační sezóny ztratila středně zatížená půda 75% C_{org} naakumulovaného během zimního období. POH bohatá na aromatické sloučeniny byla transformována na POH obohacenou o N- a P-sloučeniny. Výsledky této studie naznačily, že stabilita změn v mikrobiálních společenstvech způsobená přezimováním skotu závisela na stabilitě kvantitativních i kvalitativních změn v SOM zatížených půd.

IV

Jirout, J., Šimek, M., Elhottová, D. (*submitted manuscript*). The response of soil fungal communities to the disturbing impact of overwintering beef cattle. *Soil Biology and Biochemistry*.

Jiří Jirout was responsible for soil sampling, sample preparations, DNA and fungi isolation, and assembling of data including preparation of the manuscript.

Následující pasáž o rozsahu 24 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena v archivovaném originále disertační práce uloženém na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Abstract: Overwintering cattle outdoors causes soil surface disturbance, substantial increases of soil N_{tot}, C_{org}, and P and a shift in pH to alkaline levels. Since fungi predominate in unfertilized soils with acidic pH and have filamentous hyphae, we hypothesized that changes caused by overwintering cattle outdoors (trampling, excreta returns, and changes in soil chemistry) will lead to suppressed species richness, lower biomass, and alter the structure of fungal communities. The research was conducted on an upland pasture used for overwintering cattle more than 10 years, where the gradient of cattle impact on soil has been well developed. Both culture-dependent and -independent methods were used for the determination of either fungal species composition (cultivation; DGGE) or biomass (numbers of CFU; concentration of fungal PLFA marker 18:2ω6,9). Three soils differing in cattle impact [no- (C), moderate- (M) and severe (S)] were investigated during three subsequent years. In addition, the DGGE analysis of soils was completed by comparison with fresh cattle dungs. The composition of fungal communities showed significantly higher richness and a substantial shift in species composition in soils of cattle-impacted sites (S, M) in comparison to the non-impacted site (C). From the total number of 41 isolated fungal species, 85 % were isolated from S and M soils, whereas only 15 % were found in the C soil. The number of separated DGGE bands was significantly higher in S (30.67 ± 1.63; mean ± SD) and M (25.50 ± 1.64) soils than in the C soil (19.33 ± 1.75). Sequencing of typical bands revealed common fungal genera - *Alternaria*, *Penicillium*, *Fusarium*, *Rhizopus*, *Isaria*, and *Metarhizium*. Profiles of the S soil were enriched by bands of rumen-born anaerobic fungi (*Neocallimastix*, *Cyllamyces*) occuring mainly in profiles of excrements, where relatively low band richness (14.33 ± 1.15) was observed. Soil N_{tot} and C_{org} were evaluated as the parameters significantly influencing biomass as well as composition of the fungal community. The increase in N_{tot} and C_{org} resulted in 2 to 3 fold increases of fungal biomass in the S and M soils, contrary to the C soil. The species composition as well as diversity of both cultivable and complex fungal communities were higher in cattle impacted soils, contrary to the non-impacted parts of the cattle overwintering area.

Abstrakt: Venkovní přezimování skotu způsobuje narušení povrchu půdy a podstatné zvýšení množství N_{tot}, C_{org}, a P i zvýšení pH do zásadité oblasti. Jelikož houby převládají v nehnojených půdách s kyselou reakcí a mají vláknité hyfy, předkládali jsme, že změny způsobené skotem na zimovišti (sešlap, depozice exkrementů a změny v chemismu půdy) negativně ovlivňují biomasu, druhovou bohatost i složení společenstev hub. Výzkum byl prováděn na podhorské pastvině využívané přes 10 let pro přezimování skotu, kde se vyvinul specifický gradient zátěže skotem, se nachází na podhorské pastvině a slouží jako zimoviště skotu. Kultivačně závislé i nezávislé mikrobiologické metody byly použity pro stanovení druhového složení (kultivace, DGGE) a biomasy hub (počty CFU, koncentrace houbové PLFA 18:2ω6). Tři půdy s různou intenzitou zátěže skotem (neovlivněná, středně a silně ovlivněná) byly studovány během tříletého experimentu. Navíc byly DGGE analýzy půd porovnány se vzorky z čerstvého exkrementu. Společenstva hub v S a M půdách měla významně vyšší diverzitu a byly zde pozorovány podstatné změny v druhovém složení oproti neovlivněné půdě. Z celkového počtu 41 izolátů, 85 % bylo pozorováno v ovlivněných půdách, zatímco jen 15 % bylo nalezeno v kontrolní neovlivněné půdě. Počet separovaných fragmentů při DGGE analýze bylo významně vyšší v silně (30,67 ± 1,63; průměr ± SD) a středně (25,50 ± 1,64) ovlivněné půdě než v kontrolní půdě (19,33 ± 1,75). Sekvenováním typických fragmentů byly nalezeny běžné rody půdních hub - *Alternaria*, *Penicillium*, *Fusarium*, *Rhizopus*, *Isaria* a *Metarhizium*. Profily půdy S byly obohaceny o fragmenty anaerobních hub z bachoru (*Neocallimastix*, *Cyllamyces*), které se objevily zejména v profilech exkrementů, které vykazovaly nejnižší bohatost fragmentů (14,33 ± 1,15). Množství N_{tot} a C_{org} v půdě byly vyhodnoceny jako parametr významně ovlivňující biomasu i složení společenstva hub. Vyšší množství N_{tot} a C_{org} vedlo k dvoj- až trojnásobnému zvýšení biomasy hub v půdě S, resp. M, oproti půdě C. Složení a diverzita kultivovatelného i celkového společenstva byly vyšší v půdách ovlivněných skotem, v porovnání s neovlivněnou částí zimoviště skotu.

V

Jirout, J., Šimek, M., Elhottová, D. (*submitted manuscript*). The fungal contributors to the emissions of nitrous oxide (N_2O) from cattle overwintering area. Climatic Change

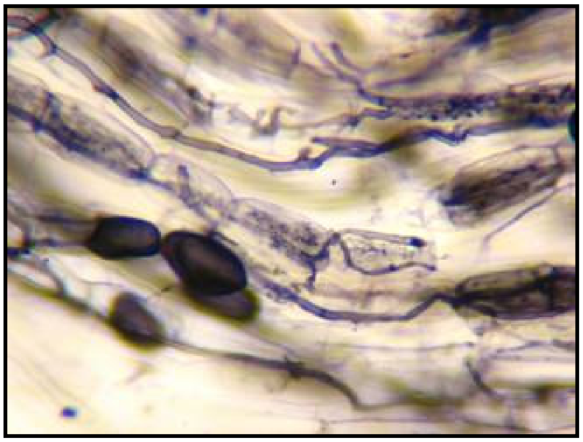
Jiří Jirout was responsible for soil sampling, isolation of fungi, preparation and maintaining of experiments, and assembling of data including preparation of the manuscript.

Následující pasáž o rozsahu 13 stran obsahuje skutečnosti chráněné autorskými právy a je obsažena v archivovaném originále disertační práce uloženém na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích.

Abstract: Soil microscopic fungi, mainly isolated from arable and forest soils, have been suggested as producers of nitrous oxide (N_2O). The aim of this paper was to screen the denitrification potential of microscopic fungi originating in the pasture soil of a cattle overwintering area. In total 36 fungal species from 11 genera were isolated during a 2-year study, and the production of N_2O under laboratory conditions was confirmed in 23 species (64%). Species belonging to the genera *Fusarium*, *Penicillium, Pseudallescheria, Acremonium,* and *Eurotium* were found to be the most active N_2O-producers. Their N_2O production amounted to ca 91% of the sum of N_2O produced by all 23 positive strains. Three different N_2O production patterns and wide production rates ranging from 1 to 150 µg $N\text{-}N_2O$ d^{-1} per flask, were observed, resulting in the transformation of 0.2 to 18.4% of the initial $N\text{-}NO_2^-$ present in the cultivation medium. There is clearly a wide range of common fungi occurring in pasture soil of the cattle overwintering area which have the ability to produce significant amounts of N_2O in laboratory conditions. The environmental consequences of this finding are briefly discussed.

Abstrakt: Půdní mikroskopické houby, většinou izolované z orné a lesní půdy, jsou považovány za producenty oxidu dusného (N_2O). Cílem tohoto článku bylo prozkoumat denitrifikační potenciál mikroskopických hub izolovaných z půdy pastviny, užívané pro přezimování skotu. Během dvouletého výzkumu bylo izolováno celkem 36 druhů hub z 11 rodů. Schoopnost produkce N_2O byla potvrzena u 23 druhů (64%). Druhy náležející k rodům *Fusarium*, *Penicillium, Pseudallescheria, Acremonium* a *Eurotium* vykázaly nejvyšší produkci N_2O. Jejich společná produkce N_2O představovala cca 91% celkové produkce N_2O všech 23 pozitivních kmenů. Byla pozorována tři různá schémata a široké rozmezí rychlostí produkce N_2O (od 1 do 150 µg $N\text{-}N_2O$ d^{-1} na láhev), z čehož vyplývá transformace 0,2 až 18,4% počátečního množství $N\text{-}NO_2^-$ obsaženého v kultivačním médiu do formy $N\text{-}N_2O$. Tento článek ukázal, že velké množství běžných půdních hub, vyskytujících se v pastevní půdě, má schopnost produkovat významná množství N_2O za laboratorních podmínek. Studie diskutuje význam těchto zjištění pro studium životního prostředí.

VI



Jirout, J., Tříska, J., Růžičková, K., Elhottová, D. 2009. Disturbing impact of outdoor cattle husbandry on community of arbuscular mycorrhizal fungi in upland pasture soil. Communications in Soil Science and Plant Analysis 40: 736–745

Jiří Jirout was responsible for soil sampling, phytocoenological analysis, sample preparations, analyses of mycorrhizal infectivity, and assembling of data including preparation of the manuscript

Abstract: The aim of the pilot study was to describe the impact of outdoor cattle husbandry on the communities of arbuscular mycorrhizal fungi on the overwintering upland pasture (South Bohemia, Czech Republic). We selected three sites with severe, moderate, and light (control) impact intensity. Roots of plant species with a plant cover area > 5% were collected at each site. The ratio of nonmycorrhizal plants species decreased with the decreasing impact of cattle. The highest mycorrhizal colonization was found at the control site on a level of the screened plant community (78.57% of root length) as well as on a species level (97.78% of root length of *Plantago major* L.). At the severely impacted site, 31.67%and 35.56% of root colonization was found on community and species level (*Plantago major* L.), respectively. Similar results were found also in the length of extraradical mycelium and arbuscules numbers.

Abstrakt: Cílem studie bylo popsat vliv venkovní pastvy skotu na společenstva arbuskulárních mykorrhizních hub na podhorské pastvině využívané jako zimoviště skotu (jižní Čechy, Česká republika). Vybrali jsme tři stanoviště s vysokou, střední a nízkou (kontrolní) intenzitou zátěže. Kořeny rostlin s pokryvností vyšší než 5% byly odebírány na každém stanovišti. Podíl nemykorrhizních rostlin se snižoval s klesající intenzitou zátěže skotem. Nejvyšší mykorrhizní kolonizace byla nalezena na kontrolním stanovišti jak na úrovni celého rostlinného společenstva (78,57% délky kořenů), tak na úrovni jednotlivých druhů (97,78% délky kořenů *Plantago major* L.). Na vysoce zatíženém stanovišti byla nalezena 31,67% a 35,56% kolonizace kořenů na úrovni společenstva, resp. rostlinných druhů (*Plantago major* L.). Podobné výsledky byly nalezeny také pro délku mimokořenového mycelia a množství arbuskulů v kořenech.

LIST OF PUBLICATIONS:

IF Journals:

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CURRICULUM VITAE

JIŘÍ JIROUT

Date and place of Birth: 22. 06. 1982 in Plzeň, Czech Republic

Nationality: Czech Republic

Gender: Male

Education:

1997 - 2001: Grammar school, Plzeň, Czech Republic

2001 - 2004: Bc. (BSc.) in Biology, University of South Bohemia in České Budějovice, Faculty of Science; Thesis title: "Decomposition rate of oak and beech litter in dependence on communities of soil micromycetes and altitude vegetation steps".

2004 - 2006: Mgr.(MSc.) in Ecology with specialization on soil biology, University of South Bohemia in České Budějovice, Faculty of Science; Thesis topic: "Changes in the community of soil saprotrophic micromycetes in conditions of the simulated shift of vegetation zones".

2006 - 2010: Ph.D. studies in Ecosystem Biology, University of South Bohemia in České Budějovice, Faculty of Science, Department of Ecosystem Biology.

Professional experience:

2006 – 2008: **Technician –** BC AS CR, v.v.i., Institute of Soil Biology.

From 2008: **Research and developmental worker -** BC AS CR, v.v.i., Institute of Soil Biology

2010 – Training in maintenance of GC and GC-MSD, HPST, s.r.o., Praha

Study Stays:

2006 – 10 days at University of Groningen, Netherlands

2007 – 7 days at RISSAC, HAS, Budapest, Hungary
10 days at ETH Zurich, Institute of Plant Sciences, Eschikon, Switzerland

2008 – 14 days at French-German summer school: Functions of microbial communities in soils: impact of anthropisation and sustainable use, Nancy, France

2008 and **2009 –** 2 times 14 days at the University of Florence, Italy

Research interests:

- Soil fungal community structure, composition, diversity
- Genotyping methods in study of structure and function of soil fungal community
- Study of microbial communities *in situ* (lipidic biomarkers techniques)
- Study of arbuscular mycorrhizae in the soil.