PALACKÝ UNIVERSITY IN OLOMOUC

FACULTY OF SCIENCE
Department of Ecology and Environmental Sciences

PAULUS ASHILI

IMPACT OF UNGULATE GRAZING TO LEAF LITTER PALATABILITY FOR DETRITOVORS

Master thesis
In
Ecology and Environmental Protection

Supervisor: Mgr. & Mgr. Ivan H. Tuf, Ph.D.

Olomouc 2008

Declaration

I hereby declare that this thesis is my own work and that all the sources i have used or quoted have been acknowledged by means of complete references.

.....	.....
Paulus Ashili	Date

Acknowledgements

First of all, I would like to express my gratitude to my thesis supervisor Mgr. & Mgr. Ivan H. Tuf, Ph.D, for supervising me, great help he gave me during my research and for the opportunity he offered me to participate in different conferences. Thank goes also to his wife Mgr. Jana Tufová for helping me during laboratory experiments as well as determination of millipedes and isopods.

I would also like to express my sincere thanks to my external supervisor RNDr. Karel Tajovský**,** CSc. for providing me with leaf litter and animals, my grateful thank also go to Mgr. Adam Véle for statistical assistance, RNDr. Petr Hekera, Ph.D., doc. RNDr. Martin Rulík, Ph.D. and Mrs Alena Habigerová for technical support during laboratory experiments. I would also like to acknowledge the financial support from the Czech science foundation, project No. 526/06/1348 and from the National Program of Research II. No. 2B06101, it was very much appreciated.

Finally, I wish to thank my family back home in Namibia: my parents, my brother and sister for their moral, encouragement and support during my study in the Czech Republic, last but not least I am also grateful to my classmates and all good people I have met throughout my stay in the Czech Republic especially in Olomouc, for creating such a wonderful atmosphere which made me feel at home.

Abstract

Ashili, P.: Impact of ungulate grazing to leaf litter palatability for detritovors.

The submitted thesis is composed of two manuscripts, both manuscripts dealt with the impact of ungulates grazing on litter quality and palatability for decomposer subsystems after heavily browsed tree shrubs by large herbivores.

In the first study we conducted a cross food preference using different species of millipedes as model groups of decomposers. Animals and leaf litter were collected at study sites in Křivoklástko PLA and BR. Millipedes were placed in small plastic boxes with plaster substrate and fed in preferential tests at 15 °C. Palatability of leaves from two different sites was compared. The first site was inside (*fenced*) area, protected from grazing of ungulates, the second site was a surrounding forest under high grazing pressure of deers (*grazed*). Litter leaves *Fagus silvatica* and *Carpinus betulus* were used. Differences of food intakes were evaluated by ANOVA with Tukey tests to pair difference. Consumption from individual experiments differed significantly (ANOVA, F= 9.36, P=0.0001). Millipedes preferred *Carpinus* to *Fagus*. In another experiment they preferred *fenced* leaves to *grazed* and *fresh* leaves instead of *old* leaves.

In the second study we examined the feeding preference of millipede *Leptoiulus proximus* (42 ind.) and woodlice *Porcellio scaber* (50 ind.). Animals were placed individually in small plastic boxes and fed in preferential tests. We offered them two kinds of hornbeam leaf litter (*Carpinus betulus*), one from the trees nibbled by deers (*nibbled*) and the second from the trees protected from grazing by fence (*untouched*). Although there were no significant differences between total consumption on both leaves (in grams), we found significant preference of *untouched* leaves in relative consumption (% amount of offered litter) by millipede (Tukey HSD, $F_{3,166} = 26.453$, $P < 0.00000$). Oppositely, woodlouse *P. scaber* consumed both leaves equally.

Key words: decomposers, food preference, leaf litter, millipedes, soil fauna.

Abstrakt

Ashili, P.: Vliv okusu spárkaté zvěře na chutnost listového opadu pro detritovory.

Tato práce je souborem dvou manuskriptů. Oba manuskripty se zabývají vlivem velkých herbivorů na kvalitu opadu a jeho chutnost pro subsystémy rozkladačů po intenzivním spásání rostlin velkými herbivory.

V první studii jsme prováděli křížové potravní pokusy s užitím různých druhů mnohonožek jako modelové skupiny rozkladačů. Jedinci byli nasbíráni na studované lokalitě v CHKO a Biosférické rezervaci Křivoklátsko stejně jako listový opad. Mnohonožky byly umístěny v malých plastových boxech se sádrovým substrátem a byly krmeny v preferenčních testech při teplotě 15 °C. Byla porovnávána chutnost listů ze dvou různých lokalit. První lokalita byla uvnitř (oploceného) území, chráněného před spásáním a herbivory, druhou lokalitou byl okolní les vystavený vysokému tlaku spásání velkými herbivory. Bylo použito spadané listí buku lesního (*Fagus silvatica*) a habru obecného (*Carpinus betulus)*. Rozdíly v příjmu potravy byly hodnoceny ANOVA s Tukeyho testy párových rozdílů. Konzumace se u různých experimentů významně lišila (ANOVA, F= 9.36, P=0.0001). Mnohonožky preferovaly habr obecný (*Carpinus betulus*) před bukem lesním (*Fagus silvatica*). V dalším experimentu preferovaly oplocené listy před spásanými a čerstvé listy před starými.

Ve druhé studii jsme testovali potravní preferenci mnohonožky *Leptoiulus proximus* (42 jedinci) a stínky *Porcellio scaber* (50 jedinců). Jedinci byli umístěni jednotlivě v malých plastových boxech a krmeni v preferenčním testu. Nabídli jsme dva různé druhy listí habru obecného (*Carpinus betulus*), jeden ze stromů okousaných herbivory (okousaných) a druhý ze stromů chráněných plotem před spásáním (nedotčených). Ačkoli nebyly shledány signifikantní rozdíly mezi celkovou konzumací obou druhů listí (v gramech), zjistili jsme signifikantní preferenci nedotčených listů z hlediska relativní konzumace (procentuální podíl nabízeného množství opadu) mnohonožkami (Tukey HSD, $F_{3,166} = 26.453$, $P < 0.00000$). Naproti tomu, stínka *P. scaber* konzumovala oba druhy listí stejně.

Klíčová slova: dekompozitoři, listový opad, mnohonožky, potravní preference, půdní fauna

TABLE OF CONTENTS

INTRODUCTION

1.1 General introduction

An increase in the population of large herbivores can cause critical changes in composition and forest community structure (Augustine and McNaughton 1998, Wardle et al. 2001, Pettit et al 1995). In most natural temperate systems, the actual density of large herbivores is relatively low. However, there are many areas of semi-natural or managed woodlands in Central Europe (at least in the Czech Republic), where densities of ungulates are unusually high and may have a marked impact on their environment.

The effects of herbivory by large vertebrates on forest plant community structure and function have been widely studied and analyzed (e.g. McNaughton 1984, Reimoser and Reimoser 1997, Hester et al. 2000, Kirby 2001 and Partl et al. 2002). In particular, ungulate may influence organic matter decomposition and nutrient cycling rates by changing the quality of plant litter entering the soil through both above and belowground pathway (Bardgett et al. 1998, Pastor et al. 1993, wardle et al. 2001). Harrison and Bardgett (2003) found that browsing by red deer on birch negatively affect the rates of decomposition in native regenerating woodland, and that these effects were, in part, related to changes in litter quality. Later, the same authors (Harrison and Bardgett 2004) measured the impact of red deer on soil nitrogen availability and concluded that removal of browsing over 14 years has accelerated native woodland regeneration, leading to subsequent increases in soil C- and N-mineralization. Pastor et al (1993) found that moose browsing in a boreal forest decreased soil nitrogen mineralization by promoting less palatable and less decomposable tree species. Similar patterns were also found in an oak savannah, however the reduction of a palatable legumes species by selective grazing appeared to be attributed to lower nitrogen mineralization in the grazed sites (Ritchie et al. 1998), whilst in tundra heath ecosystem, Olofsson and Oksanen (2002) showed that grazing by reindeer promoted grasses that decomposed faster than the dominant shrubs of lightly grazed sites.

Previous studies have shown that grazing induces changes in reallocation of nutrients within individual plants and indirectly stimulate soil mineralization processes (McNaughton 1979, 1983). Moreover, grazing increases root exudation in grassland plant, which in turn stimulates microbial biomass and activity in the rhizosphere

(Hamilton and Frank 2001). Grazing is also showed to affect root tissue chemistry through changes in carbon allocation and nutrient uptake (Holland and Deltling 1990)

On the other hand, large herbivores can have important direct impact on ecosystems, including the removal and consumption of herbage, trampling of soil and vegetation, and the return of excreta, carcasses which offer easily available plant nutrients and thus could increase nutrient cycling (pastor et al 1993). However, there are also important indirect effects of herbivores on both above- and belowground properties and processes, which can govern the net effect of herbivores on ecosystem form and productivity (Bardgett et al. 1998, Eardle et al. 2002).

So far I'm not aware of previous studies that have addressed the impact of ungulate on decomposer subsystem (soil fauna). However, there have been several studies dealing with the effects of ungulate activities on small mammals (Flowerdew and Ellwood 2001), birds (Fullar 2001) and aboveground invertebrates, mainly insects (e.g. Suominen et al. 1999, Feber et al. 2001, Stewart 2001, Suominen et al. 2003).

The impact of ungulates on soil invertebrates in woodlands is expected to be mainly indirect, mediated through the changes that ungulate grazing or browsing generates in the aboveground vegetation. The changes in structure, quantity and distribution of woodland understory have effects on the amount and quality of litter, the provision of microhabitats and on the microclimate, all of important implications for soil fauna composition and functioning. Nevertheless, the degree of soil invertebrate response will depend largely upon ungulate density. In addition, excessive density of ungulates could lead to more direct effects on soil fauna due to large disturbances of upper soil layers, soil compaction and production of faeces, as frequently reported from pastures (Piearce 1984, Cluzeau et al. 1992).

1.2 Ecological role of soil invertebrates

In temperate woodlands, soil animals such as millipedes, isopod and soil dwelling dipteran larvae and to some extent also earthworms are important components of forest ecosystems, they play a crucial role in transforming the above-ground litter entering the soil, distribution of organic matter within the soil, porosity, aeration and infiltration (Lavelle and Spain. 2001, Hedde et al. 2007, Bird et al. 2004, Zimmer et al. 2005). They affect microbial decomposition and humification and inoculation of litter and by grazing

on bacteria and fungi that grow on dead organic substrates (Brussaard, 1998), accelerate significantly the decomposition rate of litter material and aid to soil formation (Szabo 1974, Crawford 1992, Frouz et al. 1999, Martens et al. 2001). In addition, soil animals contribute to the stabilization and destabilization of soil organic matter (SOM) by simultaneously affecting chemical, physical as well as microbial processes over several orders of magnitude (Wolters 2000)

It is well established that soil fauna can affect soil processes directly and indirectly, direct effects result from the incorporation and redistribution of various materials (Wolters 1991), indirect effects is associated with soil invertebrates shaping the microbial community by both constructive for instance transporting of fungal spores and destructive means for example, selective reduction of viability (Verhoef and Brussaard 1990). Indeed feeding activities of soil animals contribute to availability of nutrient in soil as a result of increased microbial activity and excretion of excess ingested nutrients that are no longer required for production (Lussenshop, 1992; Verhoef and Brussaard 1990). Increase of nutrients in the soil by soil animals have been linked to increase in plants productivity, A number of studies have shown that plants grown in the presence of microbial-feeding soil animals often contain more N and P and produce more biomass than plants grown in the absence of soil animals (Bardgett and Chan, 1999, Alphei et al. 1996). Therefore, soil animals can be useful bioidicators of the effects of land management on nutrient dynamics and productivity.

Generally, studies on the relationship between litter palatability for soil animals have never been analyzed in correlation to the effects caused by herbivores, It is well established that herbivores can stimulate the production of secondary compounds such as polyphenols, alkaloids, or terpenes act on herbivores at many spatial and temporal scales within the plant (Barenbaun 1995, Agrawal at el. 2005) and may account up to 30% of leaf dry weight (Haukioja 1990). These changes in tissue chemical composition induced by herbivores may be retained after leaf senescence in leaf litter and have afterlife effects on rates of litter decomposition and nutrient cycling (Schweitzer et al. 2005, Findly et al. 1996).

Aims of this research

The objective of this study was to access whether the persisted of some biochemical changes persists through shedding of leaves and can affect litter palatability for detrivores (soil animals). This was achieved by:

- Comparing litter palatability from heavily grazed sites and enclosure sites using millipedes and isopods as a model group of detrivores.
- Using litter from different woody plant and different species of millipede and isopods.

References

Agrawal, A.A. (2005): Future directions in the study of induced plant response to herbivory. Etomologia Experimentalis et Applicata, 115: 97-105

Alphei, J., Bonkowskim, M., Scheu, S. (1996): Protozoa, Nematoda and Lumbricidae in the rhizosphere of *Hordelymus europaeus* (Poaceae): faunal interactions, response of microorganisms and effects on plant growth. Oecologia, 106: 1111-1126.

Augustine, D.J., McNaughton, S.J. (1998): Ungulate effects on the functional species composition of plant communities: herbovore selectivity and plant tolerance. Journal of Wildlife Management, 62: 1165-1183.

Bardgett, R.D., Chan, K.F. (1999): Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in Montana grassland ecosystem. Soil Biology and Biochemistry, 31: 1007-1014.

Bardgett, R.D., Wardle, D.A., Yeates, G.W. (1998): Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry, 30: 1867-1878.

Berenbaum, M.R. (1995): The chemistry of defense: theory and practice- proc. Natl Acad.Sci.92:2-8.

Bird, S.B., Coulson, R.N., Fisher, R.F. (2004): Changes in soil and litter arthropod abundance following tree harvesting and site preparation in a loblolly pine (*Pinus taeda* L.) plantation. Forestry Ecology and Management, 202: 195-208.

Brussaard, L. (1998): Soil fauna, guilds, functional groups and ecosystem processes. Applied Soil Ecology, 9: 123-135.

Cluzeau, D., Binet, F., Vertes, F., Simon, J.C., Riviere, J.M., Trehen, P. (1992): Effects of intensive cattle trampling on soil plant earthworms systems in 2 grassland types. Soil Biology and Biochemistry, 24: 1661-1665.

Crawford, C.S. (1992): Millipedes as model detritivores. Ber. nat.-med. Verein Innsbruck, Suppl. 10: 277-288.

Feber, R.E., Brereton, T.M., Warren, M.S., Oates, M. (2001): The impact of deer on woodland butterflies: the good, the bad and the complex. Forestry, 74: 271-276.

Findlay. S., Carreiro, M., Krischik, V.et al. (1996): Effects of damage to living plants on leaf litter quality. Ecol. Appl. 6:269-275

Flowerdew, J.R., Ellwood, S.A. (2001): Impact of woodland deer on small mammal ecology. Forestry, 74: 277-287.

Frouz J., Šantrůčková H., Elhottová D. (1999): The effect of bibionid larvae feeding on microbial community in litter and reconsumed excrements. Pedobiologia, 43: 221-230.

Fuller, R.J. (2001): Responses of woodland birds to increasing numbers of deer: a review of evidence and mechanisms. Forestry, 74: 289-298.

Harrison, K.A., Bardgett, R.D. (2003): How browsing by red deer impacts on litter decomposition in native regenerating woodland in the Highland of Scotland. Biology and Fertility of Soils, 38: 393-399.

Harrison, K.A., Bardgett, R.D. (2004): Browsing by red deer negatively impacts on soil nitrogen availability in regenerating native forest. Soil Biology and Biochemistry, 36: 115-126.

Haukioja, E. (1990): Induction of defenses in trees- Annu.Rev. Entom. 36:25-42.

Hedde, M., Bureau, F., Akpa-Vinceslas, Aubert, M., Decaens, T. (2007): Beech leaf degradation in laboratory experiments: effects of eight detritivorous invertebrate species. Applied Soil Ecology, 35: 291- 301

Hester, A.J., Edenius, L., Buttenschon, R.M., Kuiters, A.T. (2000): Interactions between forests and herbivores: the role of controlled grazing experiments. Forestry 73, 381-391.

Holland, E., Detling, J.K. (1990): Plants response to herbivory and belowground nitrogen cycling. Ecology, 71:1040-1049

Lavelle, P., Spain, A.V. (2001): Soil ecology. Kluwer Academic Publishers, Dordrecht.

Lussenhop, P. (1992): Mechanisms for microarthropod- microbial interactions. Advances in Ecological Research, 23: 1-33

Martens, H., Alphei, J., Schaefer, M., Scheu, S. (2001): Millipedes and earthworms increase the decomposition rate of N-15-labelled winter rape litter in an arable field. Isotopes in Environmental and Health Studies, 37: 43-51.

McNaughton, S.J. (1979): Grazing as an optimization process: grass-ungulate relationships in the Serengeti. American Naturalist, 113: 691-703

McNaughton, S.J.(1983): Compensatory plant growth as a response to herbivory. Oikos, 40: 329-336.

McNaughton, S.J. (1984): Grazing lawns: animals in herds, plant form, and co evolution. American Naturalist, 124: 863-886

McNaughton, S.J. (1998): Ungulate effects on the functional species composition of plant communities: herbovore selectivity and plant tolerance. Journal of Wildlife Management, 62: 1165-1183.

Olofsson, J. and Oksanen, L. (2002): Role of litter decomposition for the increased primary production in areas heavily grazed by reindeer: a litterbag experiment. Oikos, 96: 507-515.

Partl, E., Szinovatz, V., Reimoser, F., Schweiger-Adler, J. (2002): Forest restoration and browsing impact by roe deer. *Forest Ecology and Management 159, 87-100.*

Pastor, J.,Dewey B., Naiman , R.,McInnes, P., Cohen, Y.(1993): Moose browsing and soil fertility in the boreal forest of Isle Royale national park. Ecology, 74: 467-480.

Pettit N.E., Froend, R. and Ladd, P.G. (1995): Grazing in remnant woodland vegetation: changes in species composition and life form groups. Journal of vegetation science, 6: 121-130.

Piearce, T.G. (1984): Earthworm populations in soils disturbed by trampling. Biological Conservation, 29: 241-252.

Reimoser, F., Reimoser, S. (1997): Game damage and game benefit - objective assessment of the ungulate impact on the forest vegetation. Zeitschrift für Jagdwissenschaft 43, 186-196.

Ritchie, M.E., Tilman, D. And Knops, M.H. (1998): Herbivores effects on plant and nitrogen dynamics in oak savanna. Ecology,79: 165-177.

Schweitzer, J.A., Bailey, J.K., Hart, S.C; Wimp, G. M; Chapman, S.K and Whithan, T.G. (2005): The interaction of plant genotype and herbivory decelerate leaf litter decomposition and alter nutrient dynamics. Oikos,110: 133-14.5.

Stewart, A.J.A. (2001): The impact of deer on lowland woodland invertebrates: a review of the evidence and priorities for future research. Forestry, 74: 259-270.

Suominen, O., Danell, K., Bryant, J.P. (1999): Indirect effects of mammalian browsers on vegetation and ground-dwelling insects in an Alaskan floodplain. Ecoscience, 6: 505-510.

Suominen, O., Niemela, J., Martikainen P., Niemela, P., Kojola, I. (2003): Impact of reindeer grazing on ground-dwelling Carabidae and Curculionidae assemblages in Lapland. Ecography, 26: 503-513.

Szabó I.M. (1974): Microbial communities in forest rendzina ecosystem. Akademia Kiadó, Budapest.

Verhoef, H.A., Brussaard, L. (1990): Decomposition and nitrogen mineralization in natural and agro- ecosystem: the contribution of soil animals. Biogeochemistry, 11: 175-211.

Wardle, D.A., Barker, G.M., Yeates, G.W., Bonner, K.I., Ghani, A. (2001): Introduced browsing mammals in New Zealand natural forests: aboveground and belowground consequences. Ecological Monographs, 71: 587-614.

Wolters, V. (1991): Soil invertebrates- effects on nutrient turn over and soil structure. Pilanzenernahr Bodenkd, 154:389-402.

Wolters, V. (2000): Invertebrate control of soil organic matter stability. Biology Fertility Soils, 31:1-19.

Zimmer, M., Kautz, G., Topp, W. (2005): Do woodlice and earthworms interact synergistically in leaf litter decomposition? Functional Ecology, 19:7-16.

CHAPTER 2

Impact of ungulate grazing on trees to leaf litter palatability for millipedes (Diplopoda)

Paulus Ashili[1], Ivan H. Tuf[1*], Karel Tajovský[2], Jana Tufová[1]

[1] *Department of Ecology & Environmental Science, Palacky University Olomouc*

[2] *Institute of Soil Biology, Biology Centre AS CR, v. v. i., České Budějovice*

[*] *Corresponding author: ivan.tuf@upol.cz*

Abstract

Immobile plants use various strategies to defend themselves against herbivores. Production of some non palatable or hardly decomposable chemical components in leaves by overgrazed shrubs and trees represents one of these responses. The objective of this study was to assess whether some biochemical changes persist through shedding of leaves and can also affect litter palatability for detrivores.

Millipedes were used as a model group of decomposers for experimental study. In autumn 2006, 180 millipedes of seven species (*Glomeris conspersa, Glomeris hexasticha, Glomeris verhoeffi fagivora, Julus scandinavius, Leptoiulus proximus, Megaphyllum projectum* and *Unciger foetidus*) were collected at study sites in Křivoklátsko Protected Landscape Area (PLA) and Biosphere Reserve (BR). Millipedes were placed in small plastic boxes with plaster substrate and fed in preferential tests at 15 °C. Palatability of leaves from two different sites was compared. The first site was inside (*fenced*) area, protected from grazing of ungulates, the second site was a surrounding forest under high grazing pressure of deers (*grazed*). Litter leaves *Fagus silvatica* and *Carpinus betulus* were used. Differences of food intakes were evaluated by ANOVA with Tukey tests to pair difference. Consumption from individual experiments differed significantly (ANOVA, F= 9.36, P=0.0001). Millipedes preferred *Carpinus* to *Fagus.* In another experiment they preferred *fenced* leaves to *grazed* and *fresh* leaves instead of *old* leaves.

Key words: decomposers, leaf litter, millipedes, food preference, soil fauna

Introduction

In most natural temperate systems, the actual density of large herbivores is relatively low. However, there are many areas of semi-natural or managed woodlands in Central Europe (at least in the Czech Republic), where densities of ungulates are unusually high and may have a marked impact on their environment. Such impact may be of significance for either economic forestry or conservation reasons. In the past, majority of scientific papers has concentrated on studies of the impact of large herbivores in commercial forests. Contemporary, an increasing awareness is developing of an ungulate impact in woodlands managed for their conservation importance (Putman 1996).

Large herbivores can have important direct impact on ecosystems, including the removal and consumption of herbage, trampling of soil and vegetation, and the return of excreta. However, there are also important indirect effects of herbivores on both above- and belowground properties and processes, which can govern the net effect of herbivores on ecosystem form and productivity (Bardgett et al. 1998, Eardle et al. 2002). The effects of herbivory by large vertebrates on forest plant community structure and function have been widely studied and analysed (e.g. McNaughton 1984, Deyer et al. 1993, Reimoser and Reimoser 1997, Hester et al. 2000, Kirby 2001, Partl et al. 2002). Several studies are also available dealing with the effects of ungulate activities on small mammals (Flowerdew and Ellwood 2001), birds (Fullar 2001) and/or aboveground invertebrates, mainly insects (e.g. Suominen et al. 1999, Feber et al. 2001, Stewart 2001, Suominen et al. 2003).

There is, however, no detailed study relating ungulate activity with soil fauna. The impact of ungulates on soil invertebrates in woodlands is expected to be mainly indirect, mediated through the changes that ungulate grazing or browsing generates in the aboveground vegetation. The changes in structure, quantity and distribution of woodland understory have effects on the amount and quality of litter, the provision of microhabitats and on the microclimate, all of important implications for soil fauna composition and functioning. Nevertheless, the degree of soil invertebrate response will depend largely upon ungulate density. In addition, excessive density of ungulates could lead to more direct effects on soil fauna due to large disturbances of upper soil layers, soil compaction and/or production of faeces, as frequently reported from pastures (Piearce 1984, Cluzeau et al. 1992).

In response to ungulate grazing plants produce hardly decomposable and tough chemical components which are effective deterrents of herbivory, however, such components can persist in leaf litter and remain functional against decomposers (Schweitzer et al. 2005, Findlay et al. 1996, Grime, 1996). The aim of our study was to assess whether persisted of anti-herbivores components through shading of leaves can affect litter palatability for detrivores. We used different species of millipedes as model of decomposers.

Materials and methods

In 2005 and 2006, animals and litter were collected from Křivoklátsko Protected Landscape Area and Biosphere Reserve (central Bohemia, Czech Republic) a unique natural region in Central Europe. Millipedes originated from three sites (Červený kříž Nature Reserve, Benešův luh and Tři skalky) representing different types of woodland (subxerothermic oak, oak-hornbeam and beech forest respectively). Leaves from two species, beech *Fagus silvatica* and hornbeam *Carpinus betulus*, were collected at Benešův luh. Fenced enclosure was erected at each site in 1993 and leaves collected from this site were called fenced, while those from surrounding with high density of ungulates were called *grazed*. Leaves from all sites were collected directly from trees during autumn (*fresh* leaves) or in early spring (*old* leaves) and dried in laboratory conditions for few weeks.

For laboratory experiments, 180 millipedes of seven species (*Glomeris conspersa, Glomeris hexasticha, Glomeris verhoeffi fagivora, Julus scandinavius, Leptoiulus proximus, Megaphyllum projectum* and *Unciger foetidus*) were used as model group of decomposers. Maintenance of millipedes as well as individual experiments was carried out in thermostat under standardized conditions, 15 °C and high relative air humidity. Millipedes were placed in small plastic boxes with plaster substrate and fed in preferential tests. Palatability of leaves from two different variants was compared. Four experiments were done with different combinations of leaves. Millipedes were fed with leaves from the same tree species, but differed in origin (*grazed* vs. *fenced)* and age (*fresh* vs. *old)*. Before experiment, millipedes were starved for 2 days. After collecting of faecal pellets, millipedes were fed with 2 leaves (weighted after drying at 60 °C and remoistened in distilled water). After 4 days feeding, leaves were removed from the

boxes, over-dried at 60 °C and reweighed to determine consumption rate. Differences of food intakes were evaluated by ANOVA with Tukey tests to pair difference.

Results

Consumption from individual experiments differed significantly (Fig.1) (ANOVA, F= 9.36, P=0.0001). Consumption of *Fagus* leaves was significantly smaller than Carpinus leaves many times. In addition to these significant differences, we found significant preference of *fresh* litter to *old* litter for *Carpinus* in the fourth experiment with leaves from *grazed* site. The most interesting result is significant preference of *fresh Carpinus* leaf litter from fenced site than leaves from grazed site.

Discussion

Studies aimed at response of soil fauna to changes in herbivores pressure are rare yet. Changes in plant species composition resulting from herbivory caused changes in decomposition, nevertheless, mainly due to different microhabitat conditions in litter (Hector et al. 2000). Although Wardle et al. (2001) found impact of browsing of un-native ungulates to density of millipedes, simple manipulation with quality of litter (adding organic rest or man-made fertilization) had no effect to density of millipedes (Salamon et al. 2006).

Plant can decrease their palatability as response to herbivory pressure. Tree species differ in intensity of herbivore defence and it is known, that decomposition time of leaves of species unpalatable for herbivores (like snails) is longer (Cornelissen et al. 1999).

Overall, our experiment revealed significant differences in litter consumption. Millipedes exhibited a strong preference for hornbeam leaf litter than beech leaves. It is not surprising, because differences in palatability of leaves among tree species are well known (Lyford, 1943, Kondeva, 1980, Bano and Krishnamoorty, 1981). Similarly to our experiment, in Lyford's trials millipede *Cylindroiulus caeruleocinctus* (Wood, 1864) consumed leaves from hop hornbeam (*Ostria virginiana*) than beech (*Fagus grandifolia*) 30 times more (Lyford, 1943). Hornbeam leaves were consumed quickly than leaves of oak by millipede *Pachyiulus varius* (Fabricius, 1781) too (Kondeva, 1980). If millipedes have no choice and must eat less palatable leaves, they mature more quickly and reach maturity in lower weights (Banerjee, 1970).

More surprising is the result, that millipedes preferred *fresh* leaves than *old* ones. Several studies confirmed that palatability of leaf litter increase with time. David and Gillon (2002) found that fresh leaf litter of *Quercus ilex* was avoided while 6 months litter old was most palatable. This is caused by a high content of polyphenols and tannins, which is decreasing with time (Coulson et al. 1960, Bocock, 1964). This pattern of changes in palatability seems to be general, although Kheirallah (1979) found more complicated pattern of palatability. It was increasing during 2-3 months, decreasing to 5 months and had the second higher "wave" during 8 to 14 months, depending on species. Perhaps leaves of hornbeam have similar pattern as ash leaves, which are more tasty for millipedes in 1 month old (fresh ones) than 7 months old (old ones). In comparison, beech leaves were the most palatable 12 months after their fall. Similar finding was done in field conditions – millipedes preferred older leaves from F_X layer to F_1 layer (Gere, 1956). Similarly, Hassall et al. (1987) or Rushton and Hassall (1983) reported that isopods preferentially consumed decaying leaf litter that is characterized by reduced toughness and low contents of phenolics and other deterrent compounds (Kuiters and Sarink, 1986).

The most interesting result is significant preference of leaf from nongrazed tree (*fenced*) to *graze* one. Herbivores evokes defence of plants in different time scale – from immediate production of volatiles (Snoeren et al. 2007) to evolutionary differences in content of polyphenols and tannins (Gilbert and Bocock, 1960, Coulson et al. 1960, Bocock, 1964). The medium time scale is that known from birch (Tuomi et al. 1988), which produced leaves with higher content of phenols year after herbivores. Our results imply, that some chemicals defensive components produced by plant against herbivores are decreasing palatability for detritivores too and that millipedes can distinguish this leaves. Similarly millipedes ate paper with phenols and tannins significantly less in traits with different parts of extract of leaves (Sakwa, 1974).

Acknowledgements

The study was supported by the Czech Science Foundation, project No. 526/06/1348 and partially by the National Program of Research II. No. 2B06101. We are grateful to Adam Véle for statistical assistance.

References

Banerjee, B. (1970): Effects of unmixed and mixed leaf litter of three species of plants on the development and growth of *Polydesmus angustus* Latzel. Experientia, 26: 1403-1404.

Bano, K., Krishnamoorty, R.V. (1981): Consumatory responses of the millipede *Jonespeltis splendidus* (Verhoeff) in relation to soil organic matter. Proc. Indian Acad. Sci. (Anim. Sci.), 90: 631-640.

Bardgett, R.D., Wardle, D.A., Yeates, G.W. (1998): Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. Soil Biollogy and Biochemistry, 30: 1867-1878.

Bocock, K.L. (1964): Changes in the amounts of dry matter, nitrogen, carbon and energy in decomposing woodland leaf litter in relation to the activities of the soil fauna. The Journal of Ecology, 52: 273-284.

Cluzeau, D., Binet, F., Vertes, F., Simon, J.C., Riviere, J.M., Trehen, P. (1992): Effects of intensive cattle trampling on soil plant earthworms systems in 2 grassland types. Soil Biology and Biochemistry, 24: 1661-1665.

Cornelissen, J.H.C., Perez-Harguindeguy, N., Diaz, S., Grime, J.P., Marzano, B., Cabido, M., Vendramini, F., Cerabolini, B. (1999): Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. New Phytologist, 143, Special Issue: Variation in Leaf Structure: 191-200.

Coulson, C.B., Davies, R.I., Lewis, D.A. (1960): Polyphenols in plant, humus, and soil. I. Polyphenols of leaves, litter, and superficial humus from mull and mor sites. Journal of Soil Science, 11: 20–29.

David, J.-F., Gillon, D. (2002): Annual feeding rate of the millipede *Glomeris marginata* on holm oak (*Quercus ilex*) leaf litter under Mediterranean conditions. Pedobiologia, 46: 42-52.

Feber, R.E., Brereton, T.M., Warren, M.S., Oates, M. (2001): The impact of deer on woodland butterflies: the good, the bad and the complex. Forestry, 74: 271-276.

Findlay. S., Carreiro, M., Krischik, V.et al. (1996): Effects of damage to living plants on leaf litter quality- Ecol. Appl. 6:269-275.

Flowerdew, J.R., Ellwood, S.A. (2001): Impact of woodland deer on small mammal ecology. Forestry, 74: 277-287.

Fuller, R.J. (2001): Responses of woodland birds to increasing numbers of deer: a review of evidence and mechanisms. Forestry, 74: 289-298.

Gere, G. (1956): The examination of feeding biology and humificative function of Diplopoda and Isopoda. Acta biologica Hungarica, 6: 257-271.

Gilbert, O., Bocock, K.L. (1960): Changes in leaf litter when placed on the surface of soils with contrasting humus types. II. Changes in the Nitrogen content of oak and ash leaf litter. Journal of Soil Science, 11: 10–19.

Grime, J.P., Cornelissen, H.CThompson, K., Hodgson.J.G. (1996): Evidence of a causal connection between anti-herbivore defense and the decomposition rate of leaves. Oikos, 77: 489-494

Hassall, M., Turner, J.M., Rands,M.R.W. (1987): Effects of terrestrial isopods on the decomposition of woodland leaf litter, Oecologia,72: 597-604.

Hector, A., Beale, A.J., Minns, A., Otway, S.J. Lawton, J.H. (2000): Consequences of the reduction of plant diversity for liter decomposition: effects through litter quality and microenvironment. Oikos, 90: 357–371.

Hester, A.J., Edenius, L., Buttenschon, R.M., Kuiters, A.T. (2000) Interactions between forests and herbivores: the role of controlled grazing experiments. Forestry, 73: 381-391.

Kheirallah, A.M. (1979): Behavioural preference of Julus scandinavius (Myriapoda) to different species of leaf litter. Oikos, 33: 466-471.

Kirby, K.J. (2001): The impact of deer on the ground flora of British broadleaved woodland. Forestry, 74: 219-229.

Kondeva, E.A. (1980): Feeding activity of the millipede *Pachyjulus flavipes* (C.L. Koch, 1847) (Diplopoda, Pachyjulidae) and its role in the decomposition of leaf litter. Doklady Akademii Nauk SSSR, 254: 1511-1514.

Kuiters, A.T., Sarink, H.M. (1986): Leaching of phenolic compounds from leaf and needle litter of several deciduous and caniferous trees. Soil Biology and Biochemistry, 18: 475-480.

Lyford, W.H., Jr . (1943): The palatability of freshly fallen forest tree leaves to millipeds. Ecology, 24: 252-261.

McNaughton, S.J. (1984): Grazing lawns: animals in herds, plant form, and coevolution. American Naturalist, 124: 863-886.

Partl, E., Szinovatz, V., Reimoser, F., Schweiger-Adler, J. (2002): Forest restoration and browsing impact by roe deer. Forest Ecology and Management, 159: 87-100.

Piearce, T.G. (1984): Earthworm populations in soils disturbed by trampling. Biological Conservation, 29: 241-252.

Putman, R.J. (1996): Ungulate in temperate forest ecosystems: perspectives and recommenations for future research. Forestry Ecology management, 88: 205-214

Reimoser, F., Reimoser, S. (1997): Game damage and game benefit - objective assessment of the ungulate impact on the forest vegetation. Zeitschrift für Jagdwissenschaft 43, 186-196.

Rushton, S.P., Hassall,M, (1983): Food and feeding rate of terrestrial isopod *Armadillium vulgare*. Oecologia, 57: 415-419.

Sakwa, W.N. (1974): Consideration of the chemical basis of food preference in millipedes. Symposia of the Zoological Society of London, 32: 329-346.

Salamon, J.-A., Alphei, J., Ruf, A., Schaefer, M., Scheu, S., Schneider, K., Sühring, A., Maraun, M. (2006): Transitory dynamic effects in the soil invertebrate community in a temperature deciduos forest: Effects of resource quality. Soil Biology and Biochemistry, 38: 209-221.

Schweitzer, J.A., Bailey, J.K., Hart, S.C; Wimp, G. M; Chapman, S.K and Whithan, T.G. (2005): The interaction of plant genotype and herbivory decelerate leaf litter decomposition and alter nutrient dynamics. Oikos, 110: 133-145.

Snoeren, T.A.D., de Jong, P.W., Dicke, M. (2007): Ecogenomic approach to the role of herbivore-induced plant volatiles in community ecology. Journal of Ecology, 95: 17-26.

Stewart, A.J.A. (2001): The impact of deer on lowland woodland invertebrates: a review of the evidence and priorities for future research. Forestry, 74: 259-270.

Suominen, O., Danell, K., Bryant, J.P. (1999): Indirect effects of mammalian browsers on vegetation and ground-dwelling insects in an Alaskan floodplain. Ecoscience, 6: 505-510.

Suominen, O., Niemela, J., Martikainen P., Niemela, P., Kojola, I. (2003): Impact of reindeer grazing on ground-dwelling Carabidae and Curculionidae assemblages in Lapland. Ecography, 26: 503-513.

Wardle, D.A., Barker, G.M., Yeates, G.W., Bonner, K.I., Ghani, A. (2001): Introduced browsing mammals in New Zealand natural forests: aboveground and belowground consequences. Ecological Monographs, 71: 587-614.

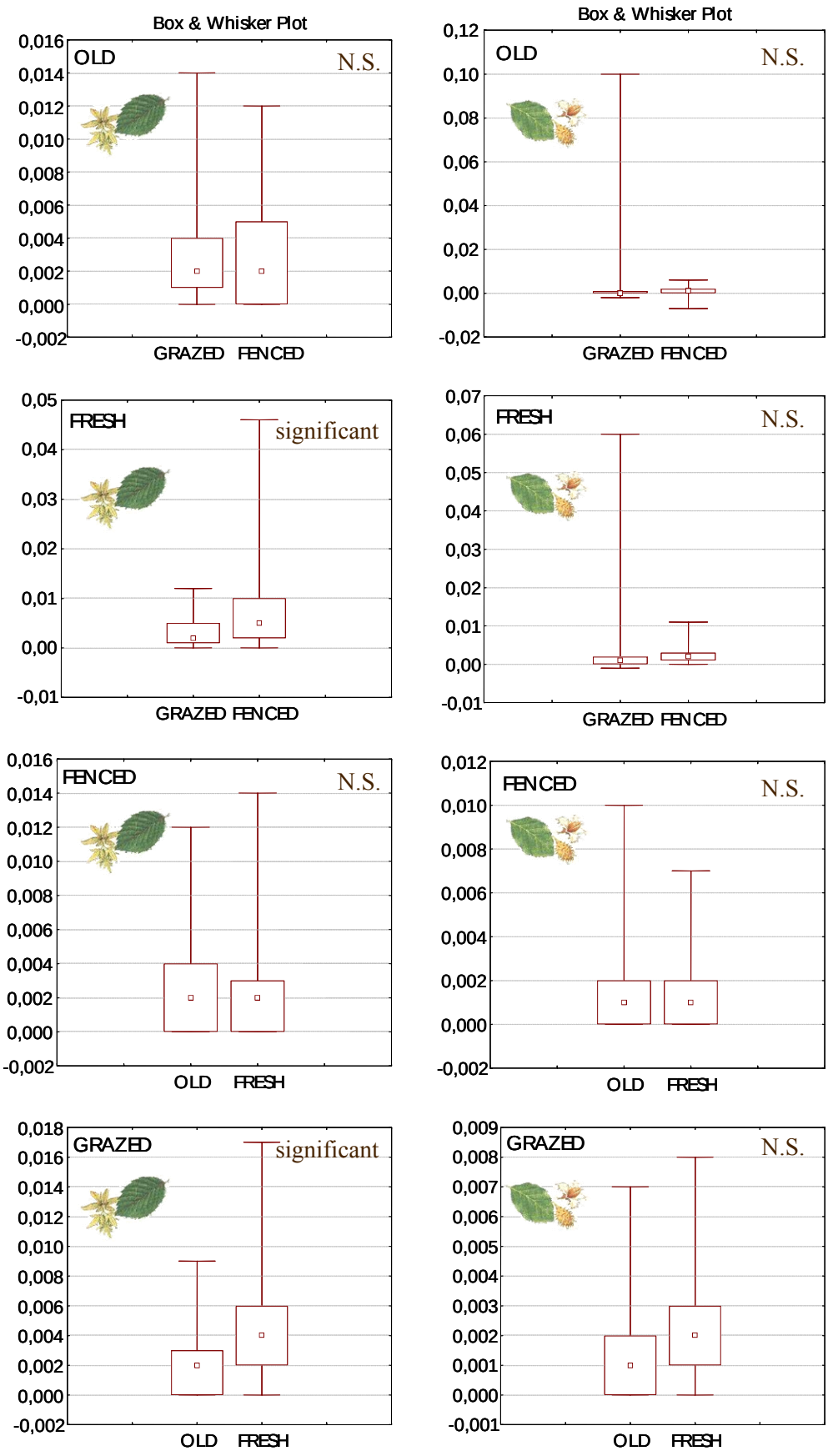


Fig.1: Food intake by millipedes in cross-preference experiments with *Carpinus* and *Fagus* leave litter, (Median, 25-75% interval and Min-Max interval).

CHAPTER 3

Who is more picky – the millipede or the woodlouse?

Millipedes are more fastidious than woodlice

Paulus Ashili[1], Ivan H. Tuf[1*], Karel Tajovský[2], Jana Tufová[1]

[1] Department of Ecology & Environmental Science, Palacky University Olomouc

[2] Institute of Soil Biology, Biology Centre AS CR, v. v. i., České Budějovice

[*] Corresponding author: ivan.tuf@upol.cz

Abstract

Immobile plants use various strategies to defend themselves against herbivores such as production of some non palatable or hardly decomposable chemical components in leaves. The objective of this study was to assess whether some biochemical changes persist through shedding of leaves and can also affect litter palatability for decomposers.

We examined the feeding preference of the millipede *Leptoiulus proximus* (42 ind.) and the woodlouse *Porcellio scaber* (50 ind.). Animals were placed individually in small plastic boxes and fed in preferential tests. We offered them two kinds of hornbeam leaf litter (*Carpinus betulus*), one from the trees nibbled by deers (*nibbled*) and the second from the trees protected from grazing by fence (*untouched*). Although there were no significant differences between total consumption on both leaves (gravimetric measurements), we found significant preference of *untouched* leaves relative consumption (percentage of offered amount of litter) by millipede (Tukey HSD, $F_{3,166} = 26.453$, $P < 0.00000$). Oppositely, woodlouse *P. scaber* consumed both leaves equally. We came to a conclusion that deer grazing had effect on litter palatability originated from grazed hornbeam shrubs for millipedes and that millipede *L. proximus* was fastidier than woodlouse *P. scaber*.

Keywords: food preference, grazing, leaf litter, decomposition, palatability, ungulates

Introduction

Many areas of semi-natural woodlands in Central Europe (particularly in the Czech Republic) are characterized by high densities of ungulates. European forests managers are concerned about the future of their forests when regeneration is completely consumed by ungulates (Jorritsma et al. 1999). Large herbivores can strongly alter vegetation composition, shifting the ecosystem into a lasting state of changed productivity (Brathen et al. 2007).

The effects of herbivores on forest community structure and functioning have been widely documented, e.g. they are known effects on organic matter decomposition and nutrient cycling rates as result of changes in litter quality entering the soil through both above and belowground pathway (Pastor et al.1993, Holland and Detling 1990, Bardgett et al. 1998, Semmartin et al 2008, Sankaran and Augustine 2004), ungulates inhibit and increase the dominant of some unpalatable plants (Gill 1992, Augustine and McNaughton 1998). Several studies are also available dealing with the effects of ungulate activities on small mammals (Flowerdew and Ellwood 2001), birds (Fullar 2001) and aboveground invertebrates, mainly insects (e.g. Suominen et al. 1999, Feber et al. 2001, Stewart 2001, Suominen et al. 2003).

Despite negative effects by large herbivores induce on ecosystem; herbivores can also play a crucial role by removal and consumption of herbage, trampling of soil and vegetation, and the return of excreta. However, there are also important indirect effects of herbivores on both above- and belowground properties and processes, which can govern the net effect of herbivores on ecosystem form and productivity (Bardgett et al. 1998).

There is, however, no detailed study relating ungulate activity with soil fauna. Plant reacts to heavy pressure of grazing through production of secondary metabolites a wide range of compounds that are hardly digestible and unpalatable to herbivores. The main objective of our study was to assess whether soil animals, such as millipedes and woodlice, are able to recognise those compounds as they persist through shading of leaves. Ability of selected millipede and woodlouse species to recognize quality of litter was compared.

Locality and methods

The main part of the material for the experimental observation was sampled in the Křivoklátsko Protected Landscape Area and Biosphere Reservation (Central Bohemia, Czech Republic) a unique natural region in Central Europe. Leaf litter was collected in the Benešův luh locality, representing oak-hornbeam woodland. A part of this woodland was fenced in 1993 to protect it from high grazing pressure of deers. Leaf litter of *Carpinus betulus* collected inside fenced area was labelled *untouched*, while that from surrounding area with nibbled trees was named as *nibbled*.

A total number of 42 specimens of the millipede *Leptoilus proximus* were collected at the same locality as leaves and in its surroundings. Specimens of the woodlouse *Porcellio scaber* (50 ind.) were collected in the city of Olomouc, because original locality did not inhabit any suitable isopod species in sufficient abundances for individual animal collection. Both species were used as model of saprophagous soil animals. Animal were placed individually in small plastic boxes with plaster substrate and fed in preferential tests. Animals were starved for a week before experiment. We offered them two kinds of leaf litter mentioned above, signed by presence/absence of basal part of footstalk. Leaves were over-dried before feeding at 60°C for 24 hrs and remoistened in distilled water. Litter weight was measured before experiment as well as after experiment to determine consumption rate. Feeding experiments lasted for four days. The experiment took place in a climate chamber under standardized conditions, at 15°C, constant dark and high air humidity (approx. 100%). Changes in dry biomass weight of consumed litter (in g) as well as consumed percentage were evaluated by Tukey HSD test to pair the differences.

Results and Discussion

There is, however, no detailed study relating ungulate activity with soil fauna. It was shown recently, that ungulates can indirectly affect decomposition and nutrient cycling by mediating feedbacks between plant communities and the decomposer subsystem (Bardgett et al. 1998, Bardgett and Wardle 2003). For example, Harrison and Bardgett (2003) found that browsing by red deer on birch affect negatively the rates of decomposition in native regenerating woodland, and that these effects were, in part, related to changes in litter quality. Later, the same authors (Harrison and Bardgett 2004) measured the impact of red deer on soil nitrogen availability and concluded that

removal of browsing over 14 years has accelerated native woodland regeneration, leading to subsequent increases in soil C- and N-mineralization.

Few studies have specifically addressed the effects of large mammals on soil microbial biomass and activity and on organic matter decomposition in woodlands (Pastor et al. 1993, Väre et al. 1996) and highlighted the idiosyncratic nature of such effects. Grime et al. (1996), Wardle et al. (1998) and Cornnelissen et at. (1999) showed that fibre content of leaves and concentration of lignins and polyphenols are negatively correlated with palatability to invertebrate herbivore and belowground decomposition rate.

We experimentally tested whether soil animals are able to recognize litter quality between *nibbled* leaf litter by ungulate grazers and *untouched* leaf litter, by using two macroarthropod groups, isopods and diplopods both considered as primary decomposers. We found no significant differences between total consumption on both leaves. However, we found significant relative preference of *untouched* leaves consumption by the millipede *L. proximus* (Tukeys HSD, $F_{3,166} = 26.453$, $P < 0.00000$). On the contrary, the woodlouse *P. scaber* consumed both leaves equally (fig.1).

Possibility of *L. proximus* to distinguish between food offers of different quality is not surprising; we found it already in millipedes before (Ashili et al. in press). As the other model animal, we used the woodlouse *P. scaber* considered to be generalist decomposers, eating dead and live plant material, roots, faeces, fungi, wood and animal remains (Lavy et al.2001, Merriam, 1970, Nair, 1976). Isopods food behaviour is well studied (e.g. Hassal and Rushton 1984, Kautz et al. 2002, Zimmer and Topp 1997, 1998, Zimmer et al.2003, Hättenschwile at al.1999; Zidar et al.2005; Teuben 1991, Kayang et al. 1996). In terms of food preference tests, isopods have been shown to be selective or discriminatory between leaves or food type which they consume (Hassal and Rushton, 1984; Neuhauser and Hartenstein 1978, Dudgeon et al. 1990). However on our experiments they tend not to selective, as they consumed equally both variants of offered leaf litter.

Evidence of selective and discriminatory feeding behaviour is assumed to be attributed mainly by the anti-herbivores protector strategies of the plants from which the litter was derived, and secondly, the influence of microbial conditioning on that litters (Hassall and Ruston 1984, Gunnarson 1987, Zimmer et al. 1996). Feeding activities of macroarthropods are known to influence microbial respiration and unlocks mineral sources such as phosphorus and nitrogen that are fixed in dead organic matter (Escher et

al. 2000). Nevertheless, isopods do not always produce the same trends (Kautz and Topp 1999). In feeding experiments with *Oniscus asellus* when offered three variants of leaf litter (sterilized, cadmium-dosed and uncontaminated or mould- covered food) animals spend significantly less time near sterilized and cadmium-dosed food than with uncontaminated or mould-covered food (Zidar et al. 2003) later the same author observed similar patterns on *Porcellio scaber* when offered two choice of leaf litter between (Cd-dosed and untreated litter), Animals visited Cd-dosed food as often as untreated food, however, they spend much less time near Cd-dosed food, later discrimination against Cd-contaminated food increased. Food experiments for *Porcellio scaber* has been widely analyzed for instance Escher et al. (2000) on a manipulated food experiments found no significant difference in consumption rate between transgenic and non transgenic maize leaf litter. Hassall et al. (2002) observed *P. scaber* spending less time on high quality food (powdered dicotyledonous leaf litter) and more on a low quality (powdered grass leaf litter). The nitrogen content of powdered dicotyledonous leaf litter was significantly higher than that of the powdered grass leaf litter but the grass litter contained significantly more carbon than the dicotyledonous plant litter. Szlavecz and Maiorana (1998) found equal consumption rate when *P. scaber* were offered artificial food supplement, one a pure protein (nitrogen) source and the other high carbohydrates and fiber.

However, in terms of selectivity and discriminatory of leaf litter by *P. scaber,* we did not observe such a trends in our experiments; *P. scaber* consumed equally both variants of offered leaf litter. Probable explanation can be an origin of tested animals; our sampled individuals were collected in urban habitats in Olomouc City where the hornbeam trees (*Carpinus betulus*) do not occur, so those woodlice had no experience with this food source. Oppositely millipedes *Leptoiulus proximus* and leaf litter were collected from the same localities with high ungulate densities and horn- beam tress are the most dominant, so millipedes probably are very well familiar with these leaf litter and as a result animal were able to identify *untouched* litter from *nibbled* leaf litter.

Feeding experiments by Pobozsný (1995) revealed significant differences in the ingested mass in the first months (December and January) when the millipede *Strongylosoma stigmatosum* were offered two variants (four broad-leaved and four coniferous tree species). However, on the followed months such a significant differences was not detected, these was probably assumed to be a “stress” caused partly by putting the animals into the pots as unusual environment to them and particularly

(which is similar to our study) offered leaf litter which was unknown to the animals. Sampled specimens of *Strongylosoma stigmatosum* were collected from the litter layer of a broad-leaved forest stand, where scots pine did not occur.

Based on our findings, we can conclude that deer grazing had effect on litter palatability for millipedes and the millipede *L. proximus* was pickier than the woodlouse *P. scaber* in identifying nibbled and untouched leaf litter.

Acknowledgements

The study was supported by the Czech Science Foundation, project No. 526/06/1348 and partially by the National Program of Research II. No. 2B06101. We are grateful to Adam Véle for statistical assistance.

References

Ashili, P., Tufová, J., Tuf, I.H., Tajovský, K. (2007): Impact of ungulate grazing on leaf litter palatability for millipedes (Diplopoda). In: Tajovský, K. (ed.): 9th Central European Workshop on Soil Zoology, Abstract book. Institute of Soil Biology, Biology Centre, ASCR, České Budějovice: 11.

Augustine, D.J., McNaughton, S.J. (1998): Ungulate effects on the functional species composition of plant communities: herbovore selectivity and plant tolerance. Journal of Wildlife Management, 62: 1165-1183.

Bardgett, R.D., Wardle, D.A. (2003): Herbivore-mediated linkages between aboveground and belowground communities. Ecology, 84: 2258-2268.

Bardgett, R.D., Wardle, D.A., Yeates, G.W. (1998): Linking above-ground and below-ground interactions: How plant responses to foliar herbivory influence soil organisms. Soil Biol. and Biochem*,* 30: 1867-1878.

Brathen, K.A., Ims, R.A., Yoccoz, N.G.,Fauchald,P.,Tveraa,T., Hausner,V.H. (2007): Induced shift in ecosystem productivity? Extensive scale effects of abundant large herbivores. Ecosystem, 10: 773-789.

Cornelissem, J.H.C., Pérez-Harguindeguy, N., Diaz, S., Grime, J.P., Marzano, B., Cabido, M., Vendramini. F., Cerabolini, B. (1999): Leaf structure and defence control litter decomposition rate across species and life form in regional floras on two continents. New phytologist, 143, 191-200.

Dudgeon D, Ma HHT, Lam PKS. 1990: Differential palatability of leaf litter of four sympatric isopods in a hong kong forest. Oecologia 84: 398-403

Escher, N., Käch, B., Nentwig, W. (2000): Decomposition of transgenic *Bacillus thuringiensis* maize by microorganisms and woodlice *Porcellio scaber* (Crustaceae: isopoda). Basic Applied Ecology 1: 161-169

Feber, R.E., Brereton, T.M., Warren, M.S., Oates, M. (2001): The impact of deer on woodland butterflies: the good, the bad and the complex. Forestry 74, 271-276.

Flowerdew, J.R., Ellwood, S.A. (2001): Impact of woodland deer on small mammal ecology. Forestry, 74, 277-287.

Gill, R.M.A. (1992): A review of damange by mammals in northern temperate forests: III. Impacts on trees and forests. Forestry, 65, 363-388

Grime,J.P., Cornelisssen, J.H.C., Thompson, K., Hodgson, J.G. (1996): Evidence of a causal connection between anti-herbivores defense and the decomposition rate of leaves. Oikos, 77,489-494.

Gunnarsson, T. (1987): Selective feeding on a maple leaf by *Oniscus asellus* (Isopoda). Pedobiologia 30: 161-165

Harrison, K.A., Bardgett, R.D. (2003): How browsing by red deer impacts on litter decomposition *Biology and Fertility of Soils* in a native regenerating woodland in the Highland of Scotland. Biology and fertility of soils 38: 393-399.

Harrison, K.A., Bardgett, R.D., (2004): Browsing by red deer negatively impacts on soil nitrogen availability in regenerating native forest. Soil Biology and Biochemistry, 36: 115-126.

Hassall, M., Rushton, S.P. (1984): Feeding behaviour of terrestrial isopods in relation to plant defences and microbial activity. Symp. Zool. Soc. Land, 53: 487-505

Hassall, M., Tuck, J.M., Smith, D.W., Gilroy, J.J., Addison, R.K. (2002): Effectc of spatial heterogeneity on feeding behaviour of *Porcellio scaber* (Isopoda: Oniscidea). European Journal of Soil Biology 38: 53-57

Hättenschwiler, S., Bühler, S., Körner., C. (1999): Quality, decomposition and isopod consumption of tree litter produced under elevated CO_2. Oikos, 2: 271-281

Holland, E., Detling, J.K. (1990): Plants response to herbivory and belowground nitrogen cycling. Ecology, 71:1040-1049

Jorristsman, I.T.M., Van Hee, A.F,M., Mhren G.M.J. (1999): Forest development in relation to ungulate grazing: a modelling approach. Forest Ecology and Management, 120: 23-34

Kautz, G., Topp, W. (1999): Acquisition of microbial communities and enhanced availability of soil nutrients by the isopod *Porcellio scaber* (Latr.) (Isopoda: Oniscidea) Biology and fertility of Soil, 31:102-107

Kayang, H., Sharma, G.D., Mishra, R.R. (1996): the influence of isopod grazing on microbial dynamics in decomposing leaf litter of Alnus nepalensis D Don. European Journal of Soil Biology, 32: 35-39

Lavy,D., Rijn Vav, M.J.,Zoomer, H.R.,Verhoef, H.R. (2001): Dietary effects on growth, reproduction, body composition and stress resistance in the terrestrial isopods *Oniscus asellus* and *Porcellio scaber.* Physiological Entomology, 26:18-25

Merriam, H.G. (1970): sensitivity of terrestrial isopod populations (Armadillidium) to food quality differences. Canadian Journal of Zoology, 49: 667-674

Nair, G.A. (1976): Food and reproduction of the soil isopod, *Porcellio leaves.* International Journal of Ecology and Environmental Science, 2:7-13

Pastor, J.,Dewey B., Naiman , R.,McInnes, P., Cohen, Y. (1993): Moose browsing and soil fertility in the boreal forest of Isle Royale national park. Ecology 74: 467-480

Pobozsny, M. (1995): Observation on the feeding behaviour of *Strongylosoma stigmatosum* (Eichwald) (Diplopoda: Polydesmoidea). Opusc. Zool. Budapest, 27-28

Sankaran, M, Augustine D., J. (2004): Large herbivores suppress decomposer abundance in semiarid grazing ecosystem. Ecology, 85: 1052-1061

Semmartin,M.,Garibaldi, L.A., Chaneton, E.J. (2008): Gazing history effects on above- and below –ground litter decomposition and nutrient cycling in two co-occuring grasses. Plant Soil, 303: 177-189

Suominen, O., Danell, K., Bryant, J.P., 1999. Indirect effects of mammalian browsers on vegetation and ground-dwelling insects in an Alaskan floodplain. Ecoscience 6, 505-510.

Suominen, O., Niemela, J., Martikainen P., Niemela, P., Kojola, I. (2003): Impact of reindeer grazing on ground-dwelling Carabidae and Curculionidae assemblages in Lapland. Ecography, 26: 503-513.

Szlavecz, K., Maiorana, C.V. (1998): Supplementary food in the diet of the terrestrial isopod *Porcellio scaber* Latr. (Isopoda: Oniscidea). Israel Journal of Zoology, 44:413-421

Teuben, A. (1991): Nutrients availability and interactions between soil arthropods and microorganisms during decomposition of coniferous litter: a mesocomsm study. Biology and Fertility of Soil, 10: 256-266

Väre, H., Ohtonen, R.,Mikkola, K. (1996): The effect of heavy grazing by reindeer in oligotrophic pine heath in northern Fennoscadian . Ecography, 19: 245-253

Wardle, D.A., Baker, G.M., Bonner, K.I.,Nicholson, K.S. (1998): Can comparative approaches based on ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems? Journal of Ecology, 86: 405-420

Zidar, P.,Kschl, U.I., Drobne, D., BožiČ, J., Štrus, J. (2003): Behavioural response in paired food choice experiments with *Oniscus asellus* (Crustacea, isopod) as an indicator of different food quality. Arh Hig Rada,Toksikol 54: 177-181

Zidar,P., Božič, J., Štrus, J. (2005): Behavioral response in the terrestrial isopod Porcellio scaber (Crustacea) offered a choice of uncontaminated and cadmium-contaminated food. Ecotoxicology, 493- 502

Zimmer, M., Kautz, G, Topp, W. (1996): Olfaction in terrestrial isopods (Crustaceae: Oniscidea): response of Porcellio scaber to odour of litter. European Journal of Soil Biology, 32:141-147

Zimmer, M., Kautz, G., Topp, W. (2003): Leaf litter-colonizing microbiota: supplementary food source or indicator of food quality for *Porcellio scaber* (Isopoda: Oniscidea)? European Journal of Soil Biology, 39: 209-216

Zimmer, M., Topp, W. (1997): Does leaf litter quality influence population parameters of the common woolouse, *Porcellio scaber* (Crustacea: Isopoda)? Biology and Fertility of Soil, 24:435-441

Zimmer, M., Topp, W. (1998): Microorganisms and cellulose digestion in the gut of the woodlouse *Porcellio scaber.* Journal of Chemical Ecology, 24(8): 1397-1408.

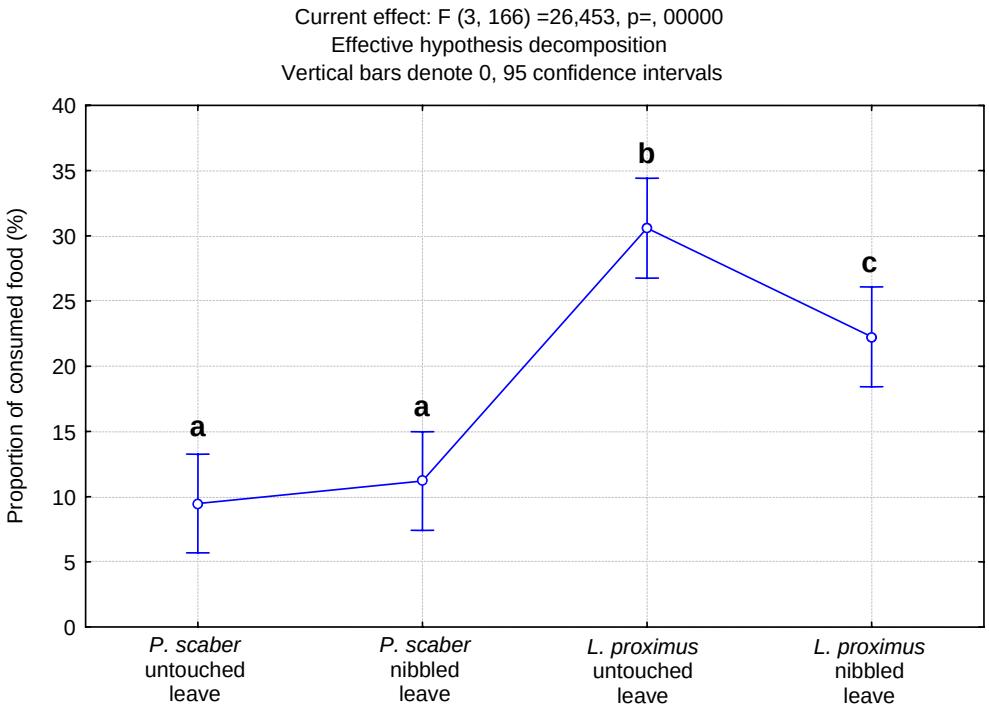


Fig.1: Feeding preferences of terrestrial isopod *P. scaber* and millipede *L. proximus* – proportional consummation of nibbled and untouched leave litter. Different letters mark significant differences.

Summary and general conclusions

The aim of this thesis was to investigate the impact of ungulate grazing on leaf litter palatability for detrivores (soil fauna) plants are immobile however, they have evolved different strategies to survive and reproduce under high densities of ungulates, productions of non palatable or hardly decomposable chemical components is one the responses by overgrazed shrubs and trees. The objective was therefore to assess whether some biochemical changes persists through shading of leaves and can affect litter palatability for detrivores too.

The thesis is composed of two manuscripts where we compared litter palatability from heavily grazed sites and enclosure sites by using two macroarthropod groups, isopods and diplopods both considered as primary decomposers and litter from different woody plant.

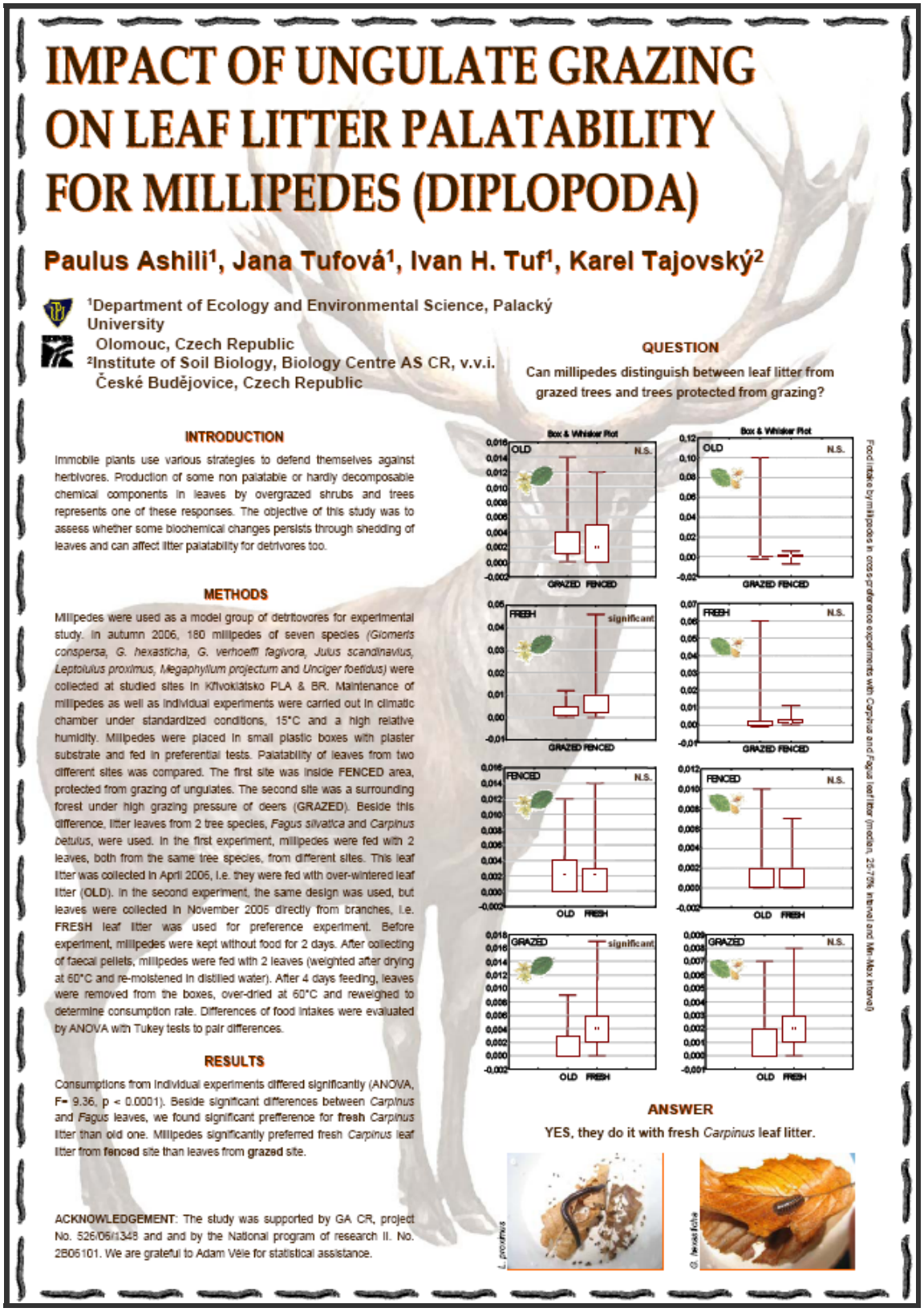
In the first manuscript we conducted a cross food preference using different species of millipedes as model groups of decomposers. Animals Millipedes were placed in small plastic boxes with plaster substrate and fed in preferential tests at 15 °C. Palatability of leaves from two different sites was compared. The first site was inside (*fenced*) area, protected from grazing of ungulates, the second site was a surrounding forest under high grazing pressure of deers (*grazed*). Litter leaves *Fagus silvatica* and *Carpinus betulus* were used. Consumption from individual experiments differed significantly (ANOVA, F= 9.36, P=0.0001). Millipedes preferred *Carpinus* to *Fagus*. In another experiment they preferred *fenced* leaves to *grazed* and *fresh* leaves instead of *old* leaves.

In the second study we examined the feeding preference of millipede *Leptoiulus proximus* (42 ind.) And woodlice *Porcellio scaber* (50 ind.). We offered them two kinds of hornbeam leaf litter (*Carpinus betulus*), one from the trees nibbled by deers (*nibbled*) and the second from the trees protected from grazing by fence (*untouched*). Although there were no significant differences between total consumption on both leaves (in g), we found significant preference of *untouched* leaves in relative consumption (% amount of offered litter) by millipede (Tukey HSD, $F_{3,166} = 26.453$, $P < 0.00000$). Oppositely, woodlouse *P. scaber* consumed both leaves equally

In conclusion, our results clearly demonstrated that high densities of ungulate grazing can have a tremendously impact on litter palatability for detrivores (soil fauna) and millipedes we more carefully in identifying grazed litter from un grazed leaf litter.

List of appendices

Appendices



IMPACT OF UNGULATE GRAZING ON LEAF LITTER PALATABILITY FOR MILLIPEDES (DIPLOPODA)

Paulus Ashili[1], Jana Tufová[1], Ivan H. Tuf[1], Karel Tajovský[2]

[1]Department of Ecology and Environmental Science, Palacký University
Olomouc, Czech Republic
[2]Institute of Soil Biology, Biology Centre AS CR, v.v.i.
České Budějovice, Czech Republic

QUESTION

Can millipedes distinguish between leaf litter from grazed trees and trees protected from grazing?

INTRODUCTION

Immobile plants use various strategies to defend themselves against herbivores. Production of some non palatable or hardly decomposable chemical components in leaves by overgrazed shrubs and trees represents one of these responses. The objective of this study was to assess whether some biochemical changes persists through shedding of leaves and can affect litter palatability for detrivores too.

METHODS

Millipedes were used as a model group of detritovores for experimental study. In autumn 2006, 180 millipedes of seven species (*Glomeris conspersa, G. hexasticha, G. verhoeffi fagivora, Julus scandinavius, Leptoiulus proximus, Megaphyllum projectum* and *Unciger foetidus*) were collected at studied sites in Křivoklátsko PLA & BR. Maintenance of millipedes as well as individual experiments were carried out in climatic chamber under standardized conditions, 15°C and a high relative humidity. Millipedes were placed in small plastic boxes with plaster substrate and fed in preferential tests. Palatability of leaves from two different sites was compared. The first site was inside **FENCED** area, protected from grazing of ungulates. The second site was a surrounding forest under high grazing pressure of deers (**GRAZED**). Beside this difference, litter leaves from 2 tree species, *Fagus silvatica* and *Carpinus betulus*, were used. In the first experiment, millipedes were fed with 2 leaves, both from the same tree species, from different sites. This leaf litter was collected in April 2006, i.e. they were fed with over-wintered leaf litter (**OLD**). In the second experiment, the same design was used, but leaves were collected in November 2006 directly from branches, i.e. **FRESH** leaf litter was used for preference experiment. Before experiment, millipedes were kept without food for 2 days. After collecting of faecal pellets, millipedes were fed with 2 leaves (weighted after drying at 60°C and re-moistened in distilled water). After 4 days feeding, leaves were removed from the boxes, over-dried at 60°C and reweighed to determine consumption rate. Differences of food intakes were evaluated by ANOVA with Tukey tests to pair differences.

RESULTS

Consumptions from individual experiments differed significantly (ANOVA, $F = 9.36$, $p < 0.0001$). Beside significant differences between *Carpinus* and *Fagus* leaves, we found significant preference for **fresh** *Carpinus* litter than old one. Millipedes significantly preferred fresh *Carpinus* leaf litter from **fenced** site than leaves from **grazed** site.

ACKNOWLEDGEMENT: The study was supported by GA CR, project No. 526/06/1348 and and by the National program of research II. No. 2B06101. We are grateful to Adam Véle for statistical assistance.

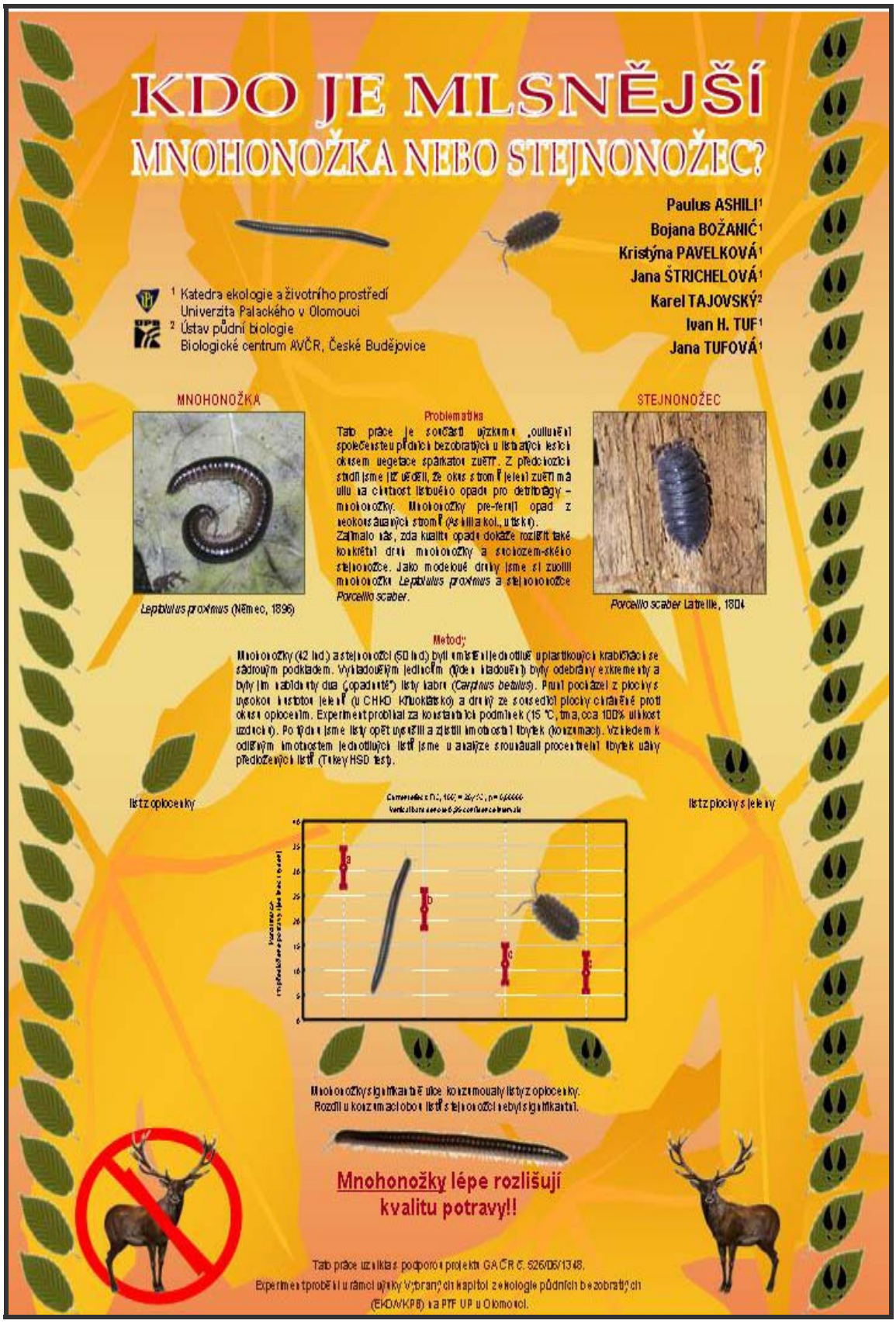
ANSWER

YES, they do it with fresh *Carpinus* leaf litter.

L. proximus

G. hexasticha

Appendix 1: Poster presentation; 9th central Europe workshop on soil zoology, České Budějovice, April 17- 20,2007



Appendix 2: Poster presentation: Zoologické dny Ceské Budějovice, 14-15.2.2008

Appendix. 3a. Example of heavily browsed shrubs at Křivoklátsko PLA and Biosphere area (2006)

Appendix. 4a. Example of heavily browsed shrubs at Křivoklátsko PLA and Biosphere area (2007)

Appendix. 4a continued

Appendix. 5a. *Leptoiulus proximus* feeding on leaf litter during Laboratory feeding experiments

Appendix.5a. continued

Glomeris sp.

Appendix. 5b. *Porcellio scaber* feeding on nibbled vs untouched leaf litter.