

Katja Ilmarinen

Defoliation and Plant–Soil  
Interactions in Grasslands











## ABSTRACT

Ilmarinen, Katja

Defoliation and plant–soil interactions in grasslands

Jyväskylä: University of Jyväskylä, 2006, 32 p.

(Jyväskylä Studies in Biological and Environmental Science,

ISSN 1456-9701; 164)

ISBN 951-39-2538-2

Yhteenveto: Defoliaatio ja kasvien ja maaperän väliset vuorovaikutukset

niittyekosysteemeissä

Diss.

To understand the structure and functioning of grassland ecosystems it is crucial to investigate how defoliation of plants (usually by herbivores or mowing) affects plants and belowground organisms and biologically regulated processes in soil. In this thesis I studied a) the impacts of defoliation on plant growth and plant-derived resources entering the soil; b) whether changes following defoliation have effects on the activity and abundances of soil organisms in different trophic groups and if these changes are long-lasting; c) the role of soil organisms in plant responses to defoliation and d) the effects of mowing on species-rich grasslands. Experiments were conducted in a field and in a greenhouse and plants were defoliated with scissors. The results show that defoliation affects the quantity and quality of plant-derived resources entering the soil. These effects, however, were highly context-dependent varying with plant species identity, the timing of defoliation in a growing season and soil nutrient status. In contrast, no widespread effects of defoliation on the components of soil food webs or soil nutrient amounts were found. Consequently, soil decomposer organisms seemed to have little importance in influencing plant nutrient acquisition, growth and recovery following defoliation under the experimental conditions I used. It was shown that defoliation can generate changes in soil that affect plant N allocation after a longer period. Moreover, by decreasing root mass and AM fungal colonization, defoliation can depress plant nutrient acquisition from soil organic matter. In species-rich grasslands mowing altered the plant community structure but did not create long-lasting changes in the grassland soil that could affect the growth of plants that subsequently colonize the site. My results emphasise, contrary to the majority of previous studies, that defoliation of plants does not always cause extensive changes in grassland soil.

Key words: Aboveground–belowground interactions; defoliation; grassland; nematodes; nitrogen; plant community; roots; soil feedback; soil food webs.

*Katja Ilmarinen, Department of Biological and Environmental Science, P.O. Box 35, FI-40014, University of Jyväskylä, Finland*

**Author's address** Katja Ilmarinen  
Department of Biological and Environmental Science  
P.O. Box 35  
FI-40014 University of Jyväskylä  
Finland  
E-mail: katja.ilmarinen@byti.jyu.fi

**Supervisors** Dr. Juha Mikola  
Department of Biological and Environmental Science  
P.O. Box 35  
FI-40014 University of Jyväskylä  
Finland

Professor David Wardle  
Department of Forest Vegetation Ecology  
Faculty of Forestry  
Swedish University of Agricultural Sciences  
SE-90183 Umeå  
Sweden

**Reviewers** Professor Søren Christensen  
Institute of Biology  
Department of Terrestrial Ecology  
Øster Farimagsgade 2D  
DK-1353 Copenhagen K  
Denmark

Dr. Christophe Robin  
UMR INPL-INRA 'Agronomie et Environnement'  
Ecole Nationale Supérieure d'Agronomie et des  
Industries Alimentaires (ENSAIA)  
BP 172  
F-54505 Vandoeuvre-les-Nancy  
France

**Opponent** Professor Richard Bardgett  
Department of Biological Sciences, I.E.N.S.  
University of Lancaster  
Lancaster, LA1 4YQ  
UK

# CONTENTS

## LIST OF ORIGINAL PUBLICATIONS

1	INTRODUCTION .....	7
1.1	The aboveground – belowground approach in grassland ecology .....	7
1.2	The effects of defoliation on plants and soil organisms .....	7
1.3	Defoliation, soil food webs and plant nutrient supply .....	9
1.3.1	Implications for the plant community structure of grasslands..	10
2	AIMS OF THE THESIS .....	11
3	MATERIALS AND METHODS .....	13
3.1	Greenhouse experiments (I, III, IV, and part of V) .....	13
3.1.1	Growth conditions in the greenhouse .....	14
3.2	The field experiment (II, and part of V).....	14
3.3	Plant and soil analyses .....	14
3.4	Data analyses.....	15
4	RESULTS AND DISCUSSION .....	17
4.1	Plant belowground responses to defoliation .....	17
4.2	Responses of soil organisms to defoliation .....	18
4.3	The role of soil organisms in nutrient dynamics and plant growth following defoliation .....	19
4.4	The role of plant – soil interactions in grassland plant community change following defoliation .....	20
5	CONCLUSIONS .....	22
	<i>Acknowledgements</i> .....	23
	YHTEENVETO.....	25
	REFERENCES.....	27



## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on five original papers, which will be referred to in the text by their Roman numerals I-V. In papers I-III and V, I took part in the original planning of the experimental design (except in I), performed the majority of the work and wrote the first draft of the manuscript, which was completed in co-operation with the other authors. In paper IV, I participated in the experimental work and the manuscript preparation.

- I Ilmarinen, K., Mikola, J., Nieminen, M. & Vestberg, M. 2005. Does plant growth phase determine the response of plants and soil organisms to defoliation? *Soil Biology and Biochemistry* 37: 433-443.
- II Ilmarinen, K., Mikola, J., Nissinen, K. & Vestberg, M. Role of soil organisms in the maintenance of semi-natural grasslands through mowing. Manuscript (Submitted).
- III Ilmarinen, K., Mikola, J. & Vestberg, M. Do interactions with soil organisms mediate grass responses to defoliation? Manuscript (Submitted).
- IV Mikola, J., Ilmarinen, K. & Vestberg, M. 2005. Long-term soil feedback on plant N allocation in defoliated grassland miniecosystems. *Soil Biology and Biochemistry* 37: 899-904.
- V Ilmarinen, K. & Mikola, J. Do soil feedbacks play a role in the effects of mowing on grassland plant community structure? Manuscript (Submitted).

# **1 INTRODUCTION**

## **1.1 The aboveground–belowground approach in grassland ecology**

Terrestrial ecosystems consist of an aboveground producer sub-system and an belowground (decomposer dominated) sub-system. However, community- and ecosystem-level processes do not occur in isolation on one side of this interface from those on the other side. Because of the variety of interactions that occur between the aboveground and belowground sub-systems, they are closely linked and therefore have to be considered in combination (Wardle 2002).

It is characteristic of grasslands that the vegetation is periodically removed by aboveground grazers or by mowing. Changes in plant aboveground production and the rate of photosynthesis caused by vegetation removal may have effects on the structure and functioning of soil food webs because the activity and abundances of soil organisms are dependent on plant belowground inputs as primary resources. Aboveground activities may therefore indirectly affect soil organisms and the processes they regulate through affecting plants. Changes in belowground food webs, in turn, may affect plant growth and resources available aboveground since soil organisms are responsible for decomposition and making nutrients available to plants (Verhoef & Brussaard 1990, Bardgett & Wardle 2003).

## **1.2 The effects of defoliation on plants and soil organisms**

Defoliation, i.e. the loss of plant shoot mass (usually by herbivores or mowing), is often manifested as changes in the growth rate and in the nitrogen (N) and carbon (C) content of plant tissues (Detling et al. 1979, Lefevre et al. 1991, Ferraro & Oesterheld 2002, Lestienne et al. 2006). Aboveground biomass

directly experiences the effects of defoliation by losing carbon and nutrients and through a decrease in photosynthesis. In the long run, both enhancement and depression in aboveground production is possible following defoliation (Milchunas & Lauenroth 1993). Belowground growth and carbohydrate reserves are also often affected by defoliation. Root mass may decrease due to the increased C allocation to re-growth of photosynthetic tissue (Caldwell et al. 1981, Ruess 1988); but increases in root mass following defoliation are also possible (Milchunas & Lauenroth 1993). Likewise, the exudation of C-rich compounds to the rhizosphere may increase (Holland et al. 1996, Mawdsley & Bardgett 1997) or decrease (Dilkes et al. 2004). The effect of defoliation on the quality (e.g. the N concentration) of plant shoots and roots is often positive (Seastedt et al. 1988, Green & Detling 2000).

The described varying and opposite plant responses following defoliation are partly explicable by the differences of individual plant species in their N and C partitioning patterns following defoliation, probably due to the different mechanisms involved in recovery from loss of foliage (Thornton & Millard 1996, Louahia et al. 1999,2000, Lestienne et al. 2006). Changes in plant growth rate and N and C metabolism can arise purely as a result of plants' physiological responses to defoliation but plant responses are, also influenced by interactions between plants and their environment. Thereby plant N and C allocation following defoliation is dependent on prevailing conditions (related to the defoliation event itself or for example to soil nutrient status) at the moment of defoliation. However, little is known about the role of these factors in mediating plant responses to defoliation.

Soil decomposer bacteria and fungi, hereby referred to as soil micro-organisms or microbes, depend on plant-derived resource inputs to meet their energy and nutrient requirements, and therefore they often respond to defoliation (Griffiths et al. 1992, Wardle 1992). The responses of soil micro-organisms and their grazers to defoliation do not show constant trends. Defoliation of plants may result in higher microbial biomass and activity in soil (Holland 1995, Mawdsley & Bardgett 1997, Guitian & Bardgett 2000); but decreases in the biomass of soil micro-organisms are also possible (Garcia and Rice 1994, Holt 1997, Mikola et al. 2001a, Sankaran & Augustine 2004), as are neutral responses (Wardle & Barker 1997, Bazot et al. 2005). Likewise, the effects of foliage removal on the abundances of microbial-feeding nematodes and microbi-detritivorous enchytraeids ranges from positive to neutral and negative (Ingham & Detling 1984, Todd et al. 1992, Mikola et al. 2001a,b, Mikola et al. 2005). Also other groups of soil organisms, such as mycorrhizal fungi and root feeding nematodes, that are more closely associated with plants, show variable responses following defoliation (Stanton 1983, Todd et al. 1992, Todd 1996, Wall-Freckman & Huang 1998, Hartley & Amos 1999, Kula et al. 2005).

Ultimately, the responses of soil organisms to defoliation are explicable by the alteration in the quantity and quality of plant-derived resources. Increased C allocation to soil by plants and higher litter and root N concentrations often explain increases in the abundances of soil organisms beneath defoliated plants;

however, this is not always the case. Knowledge about the relationship between plant-derived belowground inputs and soil organisms is still in short supply.

### **1.3 Defoliation, soil food webs and plant nutrient supply**

Defoliation affects plant growth directly by removing and damaging photosynthetic tissues and the root system. Indirect effects arise when defoliation affects biologically regulated processes in soil through its influence on the abundances of soil organisms and their trophic interactions. Soil micro-organisms are the primary decomposers of soil organic matter, but soil animals (most of which are of higher trophic levels) also have a significant role in enhancing decomposition process and in releasing nutrients immobilized in microbial tissues by grazing on microbes (Verhoef & Brussaard 1990, Setälä & Huhta 1991, Mikola et al. 2002). To date, very little is known about the relative roles of soil organisms versus other mechanisms in determining plant responses to foliage removal. Defoliation of plants might increase plant productivity if it positively affects decomposition and plant nutrient supply. It is suggested that herbivory by ruminants and clipping of foliage may increase the flux of C from roots to rhizosphere and, as a result, increase the size and activity of the biomass of soil micro-organisms (Ruess & McNaughton 1987, Holland et al. 1996). This, in turn may positively feed back to plant growth by enhancing decomposition rate of soil organic material and increasing plant nutrient supply (McNaughton et al. 1997, Bardgett et al. 1998, Hamilton & Frank 2001). In some circumstances however, increased availability of C for microbes leads to immobilization of nutrients into growing microbial biomass (Diaz et al. 1993, Jonasson et al. 1996). In such situations, microbial feeding animals have a crucial role in maintaining the availability of nutrients for plants; nutrients are released from the microbial biomass when microbial-feeding animals are consuming it (and comprising the 'soil microbial loop') (Clarholm 1985, Bonkowski et al. 2000, Paterson 2003). Accordingly, shoot N concentration often increases in grasses following defoliation, which indicates improved N acquisition (Stanton 1983, Holland & Detling 1990, Green & Detling 2000). Enhanced shoot N concentration can, on the other hand, also be due to higher relative allocation of N to shoots after defoliation (Lestienne et al. 2006) and does not necessarily reflect total plant N uptake.

It is possible that defoliation negatively affects plant nutrient acquisition and growth by decreasing root mass, but the extent of this phenomenon seems to depend on plant species identity (Thornton & Millard 1996, Hamilton & Frank 2001, Lestienne et al. 2006). The decrease in root mass following defoliation not only affects nutrient acquisition, but C availability in soil (Sankaran & Augustine 2004). Depressed plant C allocation belowground may lead to decrease in microbial biomass, decomposition and eventually, plant nutrient acquisition (Bardgett & Wardle 2003). This process is of particular

interest in relation to the conservation of species-rich semi-natural grasslands because in these habitats low nutrient availability to plants seems to be related to high plant species diversity (Collins et al. 1998, Pykälä 2005).

### **1.3.1 Implications for the plant community structure of grasslands**

Grazing and mowing are known to have an impact on the plant community composition of grasslands (Ellenberg 1988, Grime 2001). Foliage removal alters light availability within the canopy, causes flower damage and prevents litter accumulation, resulting in changes in the competitive relationships between plant species (Parr & Way 1988, Collins et al. 1998, Hulme 1996, Huhta 2001). Many studies on the impacts of grazing and mowing on grasslands have focused solely on the aboveground effects; but belowground responses can be equally interesting because soil organisms and biologically regulated processes in soil have the potential to affect the plant community composition (Klironomos 2002, De Deyn et al. 2003). In most grasslands soil nutrient supply is one of the major factors affecting plant community composition; and it is significantly affected by soil organisms (Verhoef & Brussaard 1990). It is known that plant species differ in their relative nutrient demand, nutrient acquisition strategies and the form of nutrients used (Olf et al. 1994, Dawson et al. 2003, Schimel & Bennett 2004). Thereby, it is possible that changes in soil nutrient supply after defoliation have different effects on different plant species. This could lead to changes in competitive relationships between plant species belowground and contribute to the observed changes in plant community structure aboveground.

## 2 AIMS OF THE THESIS

Links between processes occurring in aboveground and belowground compartments of grassland ecosystems have become a more actively studied topic, with increasing interest in how trophic interactions across the soil surface affect community and ecosystem level processes (Wardle et al. 2004, van Ruijven et al. 2005). Interactions between aboveground herbivores (including simulated herbivory), plants and soil micro-organisms following defoliation are much studied, whereas only a small number of studies have considered the involvement of soil animals in these interactions. However, multi-trophic perspectives regarding soil organisms following defoliation are needed because responses of primary decomposers to defoliation are often not detected because of top-down regulation of these components by their consumers (Wardle & Yeates 1993).

In my studies, I aim at clarifying the processes that follow defoliation in grasslands. To do so, it is essential to simultaneously address on plant properties, soil organisms representing different trophic groups and soil nutrient amounts. A comprehensive approach allows us to better understand plant-soil interactions and to predict the effects of management practises, such as mowing, on grassland ecology.

This thesis focuses on studying:

- \* the impacts of defoliation on plant growth and plant-derived resources entering the soil;
- \* whether changes in plant-derived resources entering the soil following defoliation have effects on the activity and abundances of soil organisms in different trophic groups and if these changes are long-lasting;

- \* the role of soil organisms in plant responses to defoliation; especially, does defoliation activate or depress the decomposer food web and thereby affect plant nutrient acquisition and growth;
- \* the effects of mowing on species-rich grasslands; especially, what is the role of soil organisms in structuring grassland plant communities and maintaining of species-rich grasslands.

## 3 MATERIALS AND METHODS

### 3.1 Greenhouse experiments (I, III, IV, and part of V)

In each experiment, soils were sieved (1 cm) and homogenized. No organisms were removed from or added to the soil, which allowed for the persistence of diverse and natural soil communities. Plants used in the experiments were typical for grasslands. When more than one species was used, the species were selected to represent different functional groups (woody plants, non-leguminous forbs, leguminous forbs and graminoids) (I, V). The aim of the first greenhouse study (I) was to examine whether plant growth phase mediates the effect of defoliation on plants and soil organisms. A three species plant community was set up and defoliated with scissors at different times during the growing season (see Table 1). Soil for this experiment originated from an abandoned grassland site. In the second greenhouse study (III) the aim was to investigate the mechanisms behind the responses of a graminoid (*Phleum pratense* L.) to defoliation, and whether these mechanisms vary in relation to soil nutrient supply and harvest time. In this study, soil was supplemented with <sup>15</sup>N enriched root litter to examine plant uptake of organic N following defoliation. Soil for this experiment originated from a cattle-pasture. Experiments IV and V were set up to study long-term effects of defoliation on soil organisms and other soil properties, and the subsequent effects on plant growth. Each of these experiments consisted of the two following phases. In the first phase either *P. pratense* (IV) or the resident vegetation in grassland sites (V) was defoliated and the growth of plants was studied following defoliation. In the second phase, soils were collected from the systems with different defoliation history and new plants were grown in these soils.



### 3.1.1 Growth conditions in the greenhouse

In the greenhouse the light from outdoors was supplemented with daylight lamps for 14 h d<sup>-1</sup>. Photosynthetic photon flux density was 75–240  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (240–800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in experiment IV) at the height of plant shoots and temperature was 18–22°C (33–38°C in experiment IV) during the light period and 14°C at night. The relative air humidity was not controlled. Soil was kept moist by regular irrigations using tap water. The pots were placed on a greenhouse table and rearranged on a regular basis to provide equal conditions for each pot.

### 3.2 The field experiment (II, and part of V)

A field trial was conducted in 2002–2004 to study the effect of mowing intensity on plants, soil organisms and soil N and P concentrations in two plant species-rich grasslands. This approach, simultaneously considering soil nutrient amounts, soil organisms and plant properties, was designed to allow insight into the mechanisms of ecological processes that follow mowing in grasslands. The experimental grasslands were originally established in 1997 in a formerly cultivated field situated on a south-facing 10° slope in Jyväskylä, Finland. Two different plant communities were established by sowing seeds of grassland plants typical of the boreal zone. Experimental plots (1m<sup>2</sup> each) on the upper part of the field were established with a species assemblage typical of dry grasslands, whereas plots situated on the lower part of the field were established with plants typical of dry-mesic grasslands. The vegetation in experimental plots was mown (with pruning clippers) either once-a-summer (in August) or twice-a-summer (in June and August), or left intact. All cuttings were instantly removed from the plots after mowing. Soil samples were taken twice each year, a month after treatments. Vegetation surveys of vascular plants in the experimental plots were performed in June each year, prior to the first mowing event. In each survey, plant species number was recorded for each plot and the cover of species in an experimental plot also recorded using an area percentage scale (0.5–100%).

### 3.3 Plant and soil analyses

Plant variables measured included dry mass of standing shoots at harvest, total shoot production (consisting of standing shoot mass at harvest plus mass of clipped shoots) (I, III, IV, V) and dry mass of roots (I–IV). Shoot and root N and C concentrations were measured with an elemental analyser (EA 1110 CHNS-O, Thermo Finnigan/CE instruments) at the Department of Biological and

Environmental Science, University of Jyväskylä (I–IV). The  $^{15}\text{N}$  concentrations of shoots and roots were analyzed by Iso Analytical Ltd, UK (III). The overall arbuscular mycorrhizal (AM) colonization rate of roots was estimated in experiments I–IV. In addition, colonization intensity and arbuscular abundance of colonized root fragments were examined in experiments II and III.

Activity and biomass of soil micro-organisms were determined using a method based on microbial basal respiration and substrate induced respiration (II, III). Prior to the microbial analyses all visible organic material particles were removed from the soil by hand. Nematodes and enchytraeids were extracted from soil sub-samples using a wet funnel method (O'Connor 1962, Sohlenius 1979) (I–IV). Total numbers of living nematodes were counted, and later using preserved samples, all individuals (or 150 individuals if the total number exceeded this) were identified to genera and allocated to trophic groups according to Yeates et al. (1993). Enchytraeids were counted live and their fresh biomass was estimated according to Abrahamsen (1973). Earthworm biomass was determined by weighing after they were removed from the soil sample and their gut cavities emptied (II). Physicochemical parameters measured from the soils were: soil water content (I–V), organic material content (II), maximum water holding capacity (II) and the concentrations of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{PO}_4\text{-P}$  (I–IV).

### 3.4 Data analyses

The treatment effects were analysed using analysis of variance (ANOVA) (I, III, V), linear mixed model (II) and t-tests (III, IV). In the cases of significant ( $P < 0.05$ ) interaction between the factors, the effects of the treatments were further examined by appropriate simple-effects analyses (Maxwell and Delaney 1990, Quinn and Keough 2003). In all analyses, the homogeneity of variances was tested using Levene's test, and when needed, the data were transformed to meet assumptions of homogeneity of variances. When these assumptions could not be met even after transformation, treatment effects were tested using non-parametric analyses according to Zar (1999). In experiments II and III associations between pairs of variables were tested using Pearson's correlation coefficient. The analyses were performed with the SPSS statistical package, except for experiment II where the mixed model analyses were carried out with the MIXED procedure of the SAS statistical package.

Plant community diversity in the grassland plots in experiment II was investigated with the Shannon diversity index,  $H' = -\sum p_i \ln p_i$ , where  $p_i$  is the proportional contribution of the species to total area cover of vegetation in a plot. Community dominance was investigated with the Shannon evenness index,  $J' = H' / \ln S$ , where  $S$  is the number of plant species in a plot (Magurran 2004).

TABLE 1 An overview of the experimental designs.

Experiment	I	II	III	IV	V
Type of experiment	Greenhouse	Field	Greenhouse	Greenhouse	Phase 1: * Phase 2: greenhouse
Experimental unit	Plant pot, size 1 l	Field plot, size 1 m <sup>2</sup>	Plant pot, size 1 l	Plant pot, size 1 l	1: * 2: plant pot
Plants	<i>Trifolium repens</i> L. <i>Plantago lanceolata</i> L. <i>Phleum pratense</i> L.	Species-rich grasslands	<i>Phleum pratense</i>	<i>Phleum pratense</i>	1: *- 2: 9 grassland species
Treatments	Defoliation Plant growth phase when defoliated	Mowing intensity Grassland type Harvest date	Defoliation Soil nutrient content Harvest date	Phase 1: defoliation Phase 2: soils with different def. history	1:* 2: Soils with different def. history
No. of defoliation events	0 or 4	0, 1 or 2 in a summer	0 or 1	1: 0 or 2 2: -	1: * 2: -
Timing of the first defoliation event	Plant age 8, 10, 14 or 20 weeks	In June and/or in August	Plant age 10 weeks	1: Plant age 17 weeks	1: *
Time between the last defoliation event and harvest	1 week	4 weeks	3 or 19 days	1: 4 weeks	

\* same as in exp. II

## 4 RESULTS AND DISCUSSION

### 4.1 Plant belowground responses to defoliation

In my studies, defoliation had a constant negative effect on living root mass. The decrease in root mass following defoliation was apparent regardless of the plant growth phase at the time of defoliation (I, II), defoliation frequency (II), soil nutrient content (III) and the plant species composition under investigation (I-IV). The results from the three-year field trial (II) did not deviate from this pattern, indicating that the negative effects of defoliation on root mass observed in the pot studies is unlikely to have resulted from experimental conditions prevailing in the greenhouse environment (McNaughton et al. 1998). The results support the view that the preferential carbon allocation by plants to their shoots following defoliation occurs at the expense of belowground allocation, often resulting in depressed root growth and an increase in root mortality (Caldwell et al. 1981, Thornton & Millard 1996, Guitian & Bardgett 2000, Mikola et al. 2001a).

I found that defoliation did not have a constant effect on root N and C concentration. In a species assemblage of three plants, in which most of the biomass consisted of a leguminous forb *T. repens*, the effect of defoliation on root N concentration and root C-to-N ratio varied from negative to positive depending on the phase of plant growth at the time of defoliation. Defoliation resulted in enhanced root N concentration when plants were defoliated in an active growth phase whereas the opposite was true when plants had well-established root and shoot systems (I). The results demonstrate that timing of defoliation within a growing season, i.e. plant age, can determine how root quality responds to defoliation. On the other hand, plant growth phase at the time of defoliation did not affect root carbon concentration (I). In species-rich grasslands mowing treatments did not cause differences in root N and C concentrations among plants (II). Whether this was an outcome of opposing responses (positive versus negative) at the individual plant level is not clear; but the results deviate from that of experiment I in that the effect of defoliation

was constant regardless of the growth phase of plants at the time of defoliation. This result might be explicable in terms of differences in the dominant plant species between the two studies; in the greenhouse study the plant community was dominated by the leguminous forb *T. repens* whereas in the field study the plots were dominated by the graminoid *Festuca ovina* L. It is possible that leguminous species are more responsive than graminoids to defoliation. However, experiment III showed that root N concentration of a graminoid (*P. pratense*) can vary depending on experimental conditions (in this case soil nutrient supply and the time for recovery) following defoliation. Root C concentration of *P. pratense* was not affected by defoliation (III).

- \* In summary, the results agree with the view that defoliation does have effects on plants and plant-derived resources entering the soil. The effects of defoliation on plant-derived resources entering the soil are not, however, easily predicted because they are significantly mediated by the experimental conditions.

## 4.2 Responses of soil organisms to defoliation

Soil food webs are generally considered to be resource (bottom-up) controlled (Wardle 1992, Wardle 2002). Accordingly, I found that, as plants aged, the root feeding nematodes became more numerous (I), suggesting that they were influenced by bottom-up regulation. Further evidence for the prevalence of resource control of soil biota was observed when the addition of organic material to soil increased the numbers of soil organisms within each of several trophic groups (III). The increase in the numbers of predatory nematodes along with root mass supports the view that soil food webs may also experience top-down regulation (Wardle & Yeates 1993) (I). On the contrary, in a study where the soil was supplemented with nutrient solution, none of the studied groups of soil organisms responded to the increase in plant biomass (III).

What became clear in my studies was that defoliation did not significantly affect the abundances of soil organisms (I-IV). The only response of soil organisms to defoliation was a decrease in AM fungal colonization, most probably because of increased photosynthate limitation given that mycorrhizal fungi require energy from plants (Caldwell et al. 1981, Gehring & Whitham 1994, Strauss & Agrawal 1999) (III). Against my expectations, and despite the well known role of bottom-up forces in regulating the abundance of soil biota, even considerable reduction of aboveground vegetation by mowing twice a summer for three growing periods did not have significant impacts on soil organisms (II). However, in this case, it should be noted that in the field there was high spatial variation in the plant species present and in soil properties.

The reasons why soil food web components did not respond to defoliation is not clear, but can be explained by several processes. Firstly, it is likely that the changes defoliation imposed on plant-derived resources entering the soil were not substantial enough to affect the populations of soil organisms. It is possible

that the decrease in living root mass following defoliation was overrun by the amount of decaying and dying roots; or that the importance of living roots as a source of C was small compared to the C already present in the soil (Mawdsley & Bardgett 1997). Secondly, it is also possible that the flow of carbon-rich substrates from roots was enhanced by defoliation (Holland et al. 1996, Paterson & Sim 2000), and that this compensated for the resources lost through root senescence. Thirdly, plants might provide root material of higher quality (higher N% or lower concentration of defensive compounds) following defoliation (Seastedt et al. 1988, Merrill et al. 1994). This could have taken place when plants were defoliated early in the growing season, at a time when defoliation resulted in senescence of a vast amount of living roots (I). It is also recognized that besides providing C to soil micro-organisms, plants compete with them for nutrients (Kaye & Hart 1997). It is thereby possible that soil micro-organisms benefited from retarded root growth following defoliation.

- \* In summary, the results demonstrate that regardless of alterations in plant-derived resources entering the soil following defoliation, widespread effects on the components of soil food webs are not necessarily found. In line with this, previous studies with coniferous forest soil have shown that the structure of soil communities is well resistant to variable environmental changes (Liiri 2001, Rantalainen 2004).

### **4.3 The role of soil organisms in nutrient dynamics and plant growth following defoliation**

Aboveground grazers may enhance grassland nutrient cycling processes, especially nitrogen mineralization, through an increase in root C exudation and stimulation of decomposer microbes (Ruess & McNaughton 1987, McNaughton et al. 1997). Accordingly, common graminoid species often display elevated shoot N concentrations following defoliation, which may reflect increased plant N supply and uptake (Wilsey et al. 1997, Green & Detling 2000, Hamilton and Frank 2001). The opposite effect may also occur if C is diverted from the system due to significant loss of foliage (Garcia & Rice 1994, Holt 1997, Sankaran & Augustine 2004). This may lead to decreased activity of soil organisms and to slower rates of nutrient mineralization and nutrient supply for plants. I explored these possibilities using a common forage grass (*P. pratense*) to see if defoliation would stimulate soil micro-organisms and their grazers, and consequently plant N uptake (III, IV). Further, I examined the role of soil organisms in determining plant community responses following defoliation using a more diverse species assemblage (I, II and V). The experiments clearly disproved the view that defoliation increases the population sizes and activity of soil micro-organisms and the activity of microbial-grazers, (i.e. the 'soil microbial loop'). Likewise, there was no evidence for enhanced C allocation to the roots and rhizosphere following defoliation. Moreover, in experiments I and

III plants did not compensate for the shoot mass lost by defoliation (but see IV) which provides further evidence for that plant nutrient acquisition was not promoted by defoliation. On the other hand, no evidence for suppressed abundances of soil organisms and soil processes was found in my studies either.

I found that defoliated plants acquired less N from soil organic matter patches (III). This indicates that defoliation and the subsequent decrease in shoot mass and AM fungi hindered the ability of plants to exploit nutrients in the soil. The results from experiment III further show that increase in shoot N concentration of the graminoid *P. pratense* was not a result of defoliation-induced positive soil feedback through a more active soil decomposer community following defoliation. Instead, it was more likely to stem from a higher relative allocation of nitrogen to shoots after defoliation. Results of experiment IV show, however, that plant N allocation patterns may be influenced by long-lasting soil changes induced by defoliation. The mechanism behind this observation, however, remained unclear because defoliation caused no changes in the abundances of soil organisms or in soil nutrient amounts.

- \* In summary, in my studies soil decomposer organisms seemed to have little importance in influencing plant nutrient acquisition and growth following defoliation. Plant physiological responses to defoliation seemed to be the main mechanisms influencing plant nitrogen dynamics.

#### **4.4 The role of plant-soil interactions in grassland plant community change following defoliation**

Foliage removal by grazing and mowing promotes conditions associated with high plant species richness and low dominance (Grime et al. 1987, Ellenberg 1988, Collins et al. 1998). Relative abundances of different plant species in a community will change because plant species differ in their abilities to take advantage of the new environmental conditions created by mowing, and because plant species differ in their resiliency to foliage damage (Caldwell et al. 1981, Huhta 2001). In the greenhouse experiment with three plant species, defoliation led to lower dominance because the contribution of the dominant species *T. repens* to total production decreased and that of *P. pratense* and *P. lanceolata* increased during the growing season (I). On the contrary, mowing did not affect species number or dominance in the studied grassland sites in the three-year field trial (V). Mowing induced changes in the relative abundances of plant functional groups: woody plants were retarded by mowing, whereas leguminous species benefited (V). These results agree with the common view that leguminous species benefit from grazing and mowing and that cutting of vegetation effectively hinders the establishment of woody plants (Collins et al. 1998, Fisher & Wipf 2002, Antonsen & Olsson 2005). The reason why *T. repens*

did not increase in abundance in the greenhouse study is not clear; but the result demonstrates that prevailing conditions, for example the identity of plant species present, may mediate the effect of mowing and grazing on plant communities. Possibly, the light intensity in the greenhouse was not sufficient for *T. repens* to fully recover from defoliation.

Changes in aboveground conditions, such as an increase in light availability in the canopy, are important factors influencing plant growth response following defoliation (Collins et al. 1998). The role of changes in soil attributes in explaining the effect of defoliation on the plant community may be important as well. My study IV, with one graminoid species, showed that clipping of plants may create long-lasting changes in grassland soil which may affect the properties of plants that subsequently colonize the site. In contrary, in experiment V, mowing history had no effect on the growth of several different grassland plant species in the greenhouse experiment despite having effects on the relative abundance of plant functional groups in the field plots. In this study, however, only plant shoot mass was measured.

- \* In summary, mowing does not seem to create changes in the soil community and soil nutrient supply that would differentially affect the growth of grassland plant species. In a three-years perspective at least, soil changes do not seem to have a significant role in determining the response of grassland plant communities to mowing.



## 5 CONCLUSIONS

The results of this thesis show that defoliation of plants does not necessarily impose widespread effects on the structure and functioning of soil food webs. Consequently, the role of soil micro-organisms and their grazers following defoliation seemed to have little importance in influencing plant nutrient acquisition, growth and recovery under the experimental conditions I used. Plant physiological responses to defoliation seemed to be the main mechanisms influencing plant nitrogen dynamics. However, some evidence was found for that plant N allocation patterns may be influenced by long-lasting soil changes induced by defoliation. The results agree with the prevailing view that plant – soil interactions are complex in nature and difficult to predict due to the wide variety of mechanisms that are involved. The responses of plants to defoliation can vary with plant species identity, plant age and soil nutrient supply. Accordingly, the activity and populations or numbers of soil organisms may increase or decrease following defoliation or, as my results emphasize, may also be neutral.

## *Acknowledgements*

Above all, my deepest gratitude goes to my supervisor Juha Mikola. Your helpfulness and commitment to this project has been exceptional. I could not have asked for more. My co-supervisor, David Wardle gave valuable comments on the thesis and the manuscripts and I am also thankful for the opportunity to join his multi-national (something like seven nationalities!) research team in Lincoln, New Zealand. Mauritz Vestberg made important contributions to this work with the mycorrhizal analyses and by kindly advising me whenever I needed help regarding mycorrhizal fungi. I express my gratitude also to Kari Nissinen for the assistance with the statistical analyses and for patiently trying to teach me the statistical way of thinking. I also thank Iuliana Popovich for identifying the nematodes. Jari Haimi and Leena Lindström offered a support which helped me get through the laborious last meters of writing the thesis.

Many other people have helped me along the way. Leena Kontiola, Titta Räisänen, Mervi Nieminen, Satu Ala-Könni, Milja Heikkinen, Sohvi Jäntti, Anu Käppi, Marko Moilanen, Suvi Pirinen, Saija Rantala and Kirsi Ruotsalainen provided assistance in the field and in the laboratory work as well as their friendship. Without your help I'd still probably be in the darkness of the Ambiotica basement washing roots and listening to radio Nova. Present and former members of the Soil Group of the University of Jyväskylä familiarized me with techniques in soil biology and were patient with my intolerance of disorder and dirt. The Ecology section had excellent facilities and a warm atmosphere for my work. Especially, thanks go to Katja O., Tanja and Louise for company during otherwise boring lunch breaks. The department staff deserves huge thanks, especially Juha and Tarmo for helping and constructing the equipment I needed; as well as our lovely office ladies and computer-Harri. The Environmental Science section kindly let me use the elemental analyser and Niina Koivula instructed me in its use. Markku Kuitunen and Päivi Tikka gave me the opportunity to use the grassland sites they have established. Roger Jones gave advice with the <sup>15</sup>N-technique. Emily Knott kindly revised the language of the summary. Thanks go to you all.

My life has been a lot more than just this work, thanks to a vast amount of friends and hobbies. I apologize that I cannot mention all my friends here by name. I thank Jyväskylän Tapiola-kuoro - maailman vanhin lapsikuoro - for relaxing company and tolerance of my absences. Special thanks go to Mira for loaning Elmeri for my company every now and then and to Marika for renting Kalle to me. Kati and Mika should be mentioned because of their warm hospitality when I have visited Tampere and the most enjoyable company: we'll keep on rocking...!

I have been blessed with a measureless amount of love and support from my mother, Terttu; my grandparents, Taina and Kalle; and Arvo. I owe my deepest gratitude to you for the facilities to grow up strong and healthy. Finally, I thank Esko; you are not only a talented scientist but a warm-hearted loving partner. I am lucky and proud to have you by my side.

This study has been financed by the Academy of Finland, University of Jyväskylä and the Finnish Cultural Foundation.

## YHTEENVETO

### Defoliaatio ja kasvien ja maaperän väliset vuorovaikutukset niittyekosysteemeissä

Niittyekosysteemeille on tunnusomaista kasvillisuuden ajoittainen laiduntaminen tai niittäminen. Versomassan poisto eli defoliaatio vaikuttaa kasvien kasvuun, kasvien resurssien allokontiin ja kasviyhteisön lajistoon. Defoliaatio voi täten epäsuorasti, kasvien kautta, vaikuttaa myös maaperän eliöyhteisöihin ja toimintaan, koska maaperän ravintoverkot ovat viime kädessä kasvilähtöisten resurssien säätelimiä. Väitöskirjassani tutkin miten defoliaatio vaikuttaa kasvien kasvuun ja maaperään kulkeutuviin kasviperäisiin resursseihin ja muuttuuko juuristossa elävien maaperäeliöiden populaatiokoot defoliaation seurauksena. Lisäksi tutkin mikä on näiden muutosten vaikutus kasvien ravinteiden saantiin, kasvuun ja kasviyhteisöjen rakenteeseen.

Tutkimukset tehtiin joko kasvihuoneessa Jyväskylän yliopiston bio- ja ympäristötieteiden laitoksella tai koealoilla kasvilajistoltaan runsaslajisilla niityillä Jyväskylässä. Kasvihuonekokeet kestivät pisimmillään yhden kasvukauden ajan ja maastokoe niityllä kesti kolme kasvukautta. Koekasveiksi valittiin tyypillisiä niittykasveja ja maanpäällistä herbivoriaa ja niittoa jäljiteltiin leikkaamalla versoja saksilla. Defoliaation seurauksia tutkittiin tarkastelemalla sekä kasvien kasvua ja kasvien laatua kuvaavia muuttujia että maaperän fysikaalisiin ja biologisiin ominaisuuksiin liittyviä muuttujia. Kasveista mitattiin verson ja juurten biomassa, hiilen ja typen pitoisuudet sekä kuinka paljon juuristossa on sienijuurta eli mykorritsaa. Leimatypen ( $^{15}\text{N}$ ) avulla selvitettiin hajotuksessa vapautuneen typen kulkeutumista kasveihin. Maaperän ominaisuuksista mitattiin kuiva-aine- ja orgaanisen aineksen pitoisuutta sekä mikrobien biomassa ja aktiivisuutta. Osassa kokeista määritettiin lisäksi maaperän typpi- ja fosforipitoisuus. Maaperäeläimistä tutkittiin sukkulamatoja ravinnonkäyttöryhmittäin, sukkulamatosukujen esiintymistä sekä änkyrimatojen ja lierojen biomassa.

Defoliaatio vähensi juurten biomassa kaikissa kokeissa riippumatta koeolosuhteista. Tulos tukee aikaisempia tutkimuksia, joissa on havaittu kasvien allokoivan hiiltä verson kasvattamiseen juurten kustannuksella korvatakseen menetettyä yhteyttävää solukkoa. Vaikka defolioidut kasvit allokoivat resursseja verson uudelleenkasvuun, jäi versomassan määrä defolioiduissa kasveissa pääsääntöisesti alhaisemmaksi kuin ei-defolioiduissa kasveissa. Defoliaation vaikutus juurten typpipitoisuuteen vaihteli tutkimusoloista riippuen. Kolmelajisen kasviyhteisön (valkoapila, heinäratamo ja timotei) defoliointi kasvun varhaisessa vaiheessa lisäsi juurten typpipitoisuutta verrattuna ei-defolioituihin kasveihin; myöhemmin kasvukaudella defolioidun kasviyhteisön juurten typpipitoisuus oli matalampi kuin ei-defolioidun kasviyhteisön. Runslajisilla niityillä defoliaation ajoituksella tai intensiteetillä

ei sen sijaan ollut vaikutusta juurten tyypipitoisuuteen. Kasvilajien tiedetään reagoivan defoliaation eri tavoin, ja kokeiden ristiriitaiset tulokset osin selittynevätkin eri osatöissä käytettyjen kasviyhteisöjen erilaisuudella. Tutkimukset myös osoittivat, että vähäravinteisessa maassa kasvavien kasvien tyypipitoisuus kasvoi defoliaation seurauksena, kun taas runsasravinteisessa maassa defoliaation vaikutus oli päinvastainen.

Vaikka defoliaatio vaikutti kasviperäisiin resursseihin maaperässä, maaperäeliöiden määrissä ei havaittu merkittäviä eroja defoliaatiokäsittelyiden välillä. Se miksi maaperäeliöt eivät reagoineet resurssien muuttumiseen, ei ole selvää, ja tulos poikkeaa valtaosasta aiempia tutkimuksia. Yllättävää oli, että maaperän ominaisuudet eivät muuttuneet edes kenttäkokeessa, jossa kasvillisuus niitettiin kaksi kertaa kesässä kolmen kasvukauden ajan. Tuloksia tarkasteltaessa on kuitenkin pidettävä mielessä kenttäkokeille tyypillinen suuri vaihtelu tausta-arvoissa. Koska defoliaatio ei vaikuttanut maaperän hajottajaverkon rakenteeseen eikä toimintaan, ei se myöskään tätä kautta nopeuttanut tai hidastanut hajotusta, ravinteiden kiertoa ja kasvien ravinteiden saantia. Timoteilla tehdyssä kasvihuonekokeessa kuitenkin saatiin viitteitä siitä, että defoliaation seurauksena maaperän ominaisuudet voivat muuttua ja vaikuttaa seuraavien kasvisukupolvien typen allokontiin. Koska defoliaatio ei kuitenkaan vaikuttanut mitattuihin muuttujiin maaperässä, esimerkiksi eliöiden esiintymiseen tai typen määrään, ilmiön takana oleva mekanismi jäi selvittämättä. Tulokset osoittivat, että kasvin typen otto saattaa heikentyä, koska defoliaatio pienentää juurimassaa ja mykorritsainfektiota.

Versomassan poiston seurauksena lajienvälisen kilpailun ja niityn kasviyhteisön rakenteen tiedetään muuttuvan. Näin kävi myös omassa tutkimuksessani, kun kolmen kasvilajin yhteisössä valkoapilan dominanssi pienentyi defoliaation seurauksena. Niityllä tehdyssä kokeessa lajimäärässä tai kasvien dominanssisuhteissa ei sen sijaan havaittu muutoksia niiton jälkeen. Niitto kuitenkin muutti toiminnallisten kasviryhmien suhteellisia runsauksia: puuvaraiset kasvit taantuivat ja palkokasvit, erityisesti apilat, lisääntyivät niiton seurauksena. Kun eri niitylajien taimia kasvatettiin eri niittokäsittelyistä peräisin olevissa maissa, havaittiin, että maan niittohistoria ei vaikuttanut taimien kasvuun. Tulos viittaa siihen, että defoliaation seurauksena maan päällä tapahtuvat muutokset, esimerkiksi lisääntyvä valon määrä, ovat maan alla tapahtuvia muutoksia merkityksellisempiä tekijöitä kasviyhteisöjen muokkaajina.

Väitöskirjatutkimukseni osoittivat, että defoliaatio vaikuttaa maaperään kulkeutuvien kasviperäisten resurssien määrään ja laatuun, mutta se, minkä suuntaisia defoliaation aikaansaamat muutokset ovat, riippuu suuresti koeoloista. Sekä kasvilaji, defoliaation ajoitus kasvukaudella että maaperän ravinnepitoisuus, vaikuttivat tutkimuksissani siihen millaisia vasteita kasveissa havaittiin defoliaation seurauksena. Sen sijaan, vasten odotuksiani, maaperän eliöyhteisöissä tai orgaanisen aineksen hajotuksessa ei tapahtunut merkittäviä muutoksia defoliaation seurauksena.

## REFERENCES

- Abrahamsen, G. 1973. Studies on body-volume, body-surface area, density and live weight of Enchytraeidae (Oligochaeta). *Pedobiologia* 13: 6-15.
- Antonsen, H. & Olsson, P. A. 2005. Relative importance of burning, mowing and species translocation in the restoration of a former boreal hayfield: responses of plant diversity and the microbial community. *J. Appl. Ecol.* 42: 337-347.
- Bardgett, R. D. & Wardle, D. A. 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* 84: 2258-2268.
- Bardgett, R. D., Leemans, D. K., Cook, R. & Hobbs, P. J. 1997. Seasonality of the soil biota of grazed and ungrazed hill grasslands. *Soil Biol. Biochem.* 29: 1285-1294.
- 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. Biochem.* 30: 1867-1878.
- Bazot, S., Mikola, J., Nguyen, C. & Robin, C. 2005. Defoliation-induced changes in carbon allocation and root soluble carbon concentration in field-grown *Lolium perenne* plants: do they affect carbon availability, microbes and animal trophic groups in soil? *Funct. Ecol.* 19: 886-896.
- Bonkowski, M., Cheng, W., Griffiths, B. S., Alphei, J. & Scheu, S. 2000. Microbial-faunal interactions in the rhizosphere and effects on plant growth. *Eur. J. Soil Biol.* 36: 135-147.
- Caldwell, M. M., Richards, J. H., Johnson, D. A., Nowak, R. S. & Dzurec, R. S. 1981. Coping with herbivory: photosynthetic capacity and resource allocation in two semiarid *Agropyron* bunchgrasses. *Oecologia* 50: 14-24.
- Clarholm, M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. *Soil Biol. Biochem.* 17: 181-187.
- Collins, S. L., Knapp, A. K., Briggs, J. M., Blair, J. M. & Steinauer, E. M. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* 280: 745-747.
- Dawson, L. A., Thornton, B., Pratt, S. M. & Paterson, E. 2003. Morphological and topological responses of roots to defoliation and nitrogen supply in *Lolium perenne* and *Festuca ovina*. *New Phytol.* 161: 811-818.
- De Deyn, G. B., Raaijmakers, C. E., Zoomer, H. R., Berg, M. P., de Ruiter, P. C., Verhoef, H. A., Bezemer, T. M. & van der Putten, W. H. 2003. Soil invertebrate fauna enhances grassland succession and diversity. *Nature* 422: 711-713.
- Detling, J. K., Dyer, M. I. & Winn, D. T. 1979. Net photosynthesis, root respiration, and regrowth of *Bouteloua gracilis* following simulated grazing. *Oecologia* 41: 127-134.
- Diaz, S., Grime, J. P., Harris, J. & McPherson, E. 1993. Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* 364: 616-617.

- Dilkes, N. B., Jones, D. L. & Farrar, J. 2004. Temporal dynamics of carbon partitioning and rhizodeposition in wheat. *Plant Physiol.* 134: 706-715.
- Ellenberg, H. 1988. *Vegetation Ecology of Central Europe* (4. edition). Cambridge: Cambridge University Press.
- Ferraro, D. O. & Oosterheld, M. 2002. Effect of defoliation on grass growth. A quantitative review. *Oikos* 98: 125-133.
- Fisher, M. & Wipf, S. 2002. Effect of low-intensity grazing on the species-rich vegetation of traditionally mown subalpine meadows. *Biol. Conserv.* 104: 1-11.
- Garcia, F. O. & Rice, C. W. 1994. Microbial biomass dynamics in tallgrass prairie. *Soil Sci. Soc. Am. J.* 58: 816-823.
- Gehring, C. A. & Whitham, T. G. 1994. Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. *Trends Ecol. Evol.* 9: 251-255.
- Green, R. A. & Detling, J. K. 2000. Defoliation-induced enhancement of total aboveground nitrogen yield of grasses. *Oikos* 91: 280-284.
- Griffiths, B. S., Welschen, R., van Arendonk, J. J. C. M. & Lambers, H. 1992. The effect of nitrate-nitrogen supply on bacteria and bacterial-feeding fauna in the rhizosphere of different grass species. *Oecologia* 91: 253-259.
- Grime, J. P. 2001. *Plant Strategies, Vegetation Processes, and Ecosystem Properties* (2. edition). Chichester: John Wiley & Sons, Ltd.
- Grime, J. P., Mackey, J. M. L., Hillier, S. H. & Read, D. J. 1987. Floristic diversity in a model system using experimental microcosms. *Nature* 328: 420-422.
- Guitian, R. & Bardgett, R. D. 2000. Plant and soil microbial responses to defoliation in temperate semi-natural grassland. *Plant Soil* 220: 271-277.
- Hamilton, E. W. & Frank, D. A. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* 82: 2397-2402.
- Hartley, S. E. & Amos, L. 1999. Competitive interactions between *Nardus stricta* L. and *Calluna vulgaris* (L.) Hull: the effect of fertilizer and defoliation on above- and below-ground performance. *J. Ecol.* 87: 330-340.
- Holland, E. A. & Detling, J. K. 1990. Plant response to herbivory and belowground nitrogen cycling. *Ecology* 71: 1040-1049.
- Holland, J. N. 1995. Effects of above-ground herbivory on soil microbial biomass in conventional and no-tillage agroecosystems. *Appl. Soil Ecol.* 2: 275-279.
- Holland, J. N., Cheng, W. & Crossley Jr, D. A. 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. *Oecologia* 107: 87-94.
- Holt, J. A. 1997. Grazing pressure and soil carbon, microbial biomass and enzyme activities in semi-arid northeastern Australia. *Appl. Soil Ecol.* 5: 143-149.
- Huhta, A.-P. 2001. Restorative mowing on semi-natural grasslands: community-level changes and species-level responses. *Acta Universitatis Ouluensis. A Scientiae Rerum Naturalium* 365: 1-45.

- Hulme, P. E. 1996. Herbivory, plant regeneration, and species coexistence. *J. Ecol.* 84: 609-615.
- Ingham, E. R. & Detling, J. K. 1984. Plant-herbivore interactions in North American mixed-grass prairie. III. Soil nematode populations and root biomass on *Cynomys ludovicianus* colonies and adjacent uncolonized areas. *Oecologia* 63: 307-313.
- Jonasson, S., Michelsen, A., Schmidt, I. K., Nielsen, E. V. & Callaghan, T. V. 1996. Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake. *Oecologia* 106: 507-515.
- Kaye, J. P. & Hart, S. C. 1997. Competition for nitrogen between plants and soil microorganisms. *Trends Ecol. Evol.* 12: 139-143.
- Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417: 67-70.
- Kula, A. A. R., Hartnett, D. C. & Wilson, G. W. T. 2005. Effects of mycorrhizal symbiosis on tallgrass prairie plant-herbivore interactions. *Ecol. Lett.* 8: 61-69.
- Lefevre, J., Bigot, J. & Boucaud, J. 1991. Origin of foliar nitrogen and changes in free amino-acid composition and content of leaves, stubble, and roots of perennial ryegrass during re-growth after defoliation. *J. Exp. Bot.* 42: 89-95.
- Lestienne, F., Thornton, B. & Gastal, F. 2006. Impact of defoliation intensity and frequency on N uptake and mobilization in *Lolium perenne*. *J. Exp. Bot.* 57: 997-1006.
- Liiri, M. 2001. Complexity of soil faunal communities in relation to ecosystem functioning in coniferous soil - A disturbance oriented study. *Jyväskylä Studies in Biological and Environmental Science* 104:1-36.
- Louahlia, S., Macduff, J. H., Ourry, A., Humphreys, M. & Boucaud, J. 1999. Nitrogen reserve status affects the dynamics of nitrogen remobilization and mineral nitrogen uptake during recovery of contrasting cultivars of *Lolium perenne* from defoliation. *New Phytol.* 142: 451-462.
- Louahlia, S., Lainé, P., Thornton, B., Ourry, A. & Boucaud, J. 2000. The role of N-remobilisation and the uptake of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  by *Lolium perenne* L. in laminae growth following defoliation under field conditions. *Plant Soil* 220: 175-187.
- Magurran, A. E. 2004. *Measuring Biological Diversity*. Oxford: Blackwell Science.
- Mawdsley, J. L. & Bardgett, R. D. 1997. Continuous defoliation of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) and associated changes in the composition and activity of the microbial population of an upland grassland soil. *Biol. Fertil. Soils* 24: 52-58.
- Maxwell, S. E. & Delaney, H. D. 1990. *Designing experiments and analyzing data: A Model Comparison Perspective*. Belmont: Wadsworth.



- McNaughton, S. J., Banyikwa, F. F. & McNaughton, M. M. 1997. Promotion of the cycling of diet-enhancing nutrients by African grazers. *Science* 278: 1798-1800.
- McNaughton, S. J., Banyikwa, F. F. & McNaughton, M. M. 1998. Root biomass and productivity in a grazing ecosystem: the Serengeti. *Ecology* 79: 587-592.
- Merrill, E. H., Stanton, N. L. & Hak, J. C. 1994. Responses of bluebunch wheatgrass, Idaho fescue, and nematodes to ungulate grazing in Yellowstone National Park. *Oikos* 69: 231-240.
- Mikola, J., Yeates, G. W., Barker, G. M., Wardle, D. A. & Bonner, K. I. 2001a. Effects of defoliation intensity on soil food-web properties in an experimental grassland community. *Oikos* 92: 333-343.
- Mikola, J., Yeates, G. W., Wardle, D. A., Barker, G. M. & Bonner, K. I. 2001b. Response of soil food-web structure to defoliation of different plant species combinations in an experimental grassland community. *Soil Biol. Biochem.* 33: 205-214.
- Mikola, J., Bardgett, R. D. & Hedlund, K. 2002. Biodiversity, ecosystem functioning and soil decomposer food webs. *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives*. In: Loreau, M., Naeem, S. & Inchausti, P. (eds.). Oxford: Oxford University Press, pp. 169-180.
- Mikola, J., Nieminen, M., Ilmarinen, K. & Vestberg, M. 2005. Belowground responses by AM fungi and animal trophic groups to repeated defoliation in an experimental grassland community. *Soil Biol. Biochem.* 37: 1630-1639.
- Milchunas, D. G. & Lauenroth, W. K. 1993. Quantitative effects of grazing on vegetation and soils over a global range of environments. *Ecol. Monogr.* 63: 327-366.
- O'Connor, F. B. 1962. The extraction of Enchytraeidae from soil. In: Murphy, P. W. (ed.). *Progress in soil zoology*. London: Butterworths, pp. 279-285.
- Olf, H., Berendse, F. & De Visser, W. 1994. Changes in nitrogen mineralization, tissue nutrient concentrations and biomass compartmentation after cessation of fertilizer application to mown grassland. *J. Ecol.* 82: 611-620.
- Parr, T. W. & Way, J. M. 1988. Management of roadside vegetation: the long-term effects of cutting. *J. Appl. Ecol.* 25: 1073-1087.
- Paterson, E. 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. *Eur. J. Soil Sci.* 54: 741-750.
- Paterson, E. & Sim, A. 2000. Effect of nitrogen supply and defoliation on loss of organic compounds from roots of *Festuca rubra*. *J. Exp. Bot.* 51: 1449-1457.
- Pykälä, J. 2005. Plant species responses to cattle grazing in mesic semi-natural grassland. *Agr. Ecosyst. Environ.* 108: 109-117.
- Quinn, G. P. & Keough, M. J. 2003. *Experimental design and data analysis for biologists*. Cambridge: Cambridge University Press.
- Rantalainen, M.-L. 2004. Sensitivity of soil decomposer communities to habitat fragmentation – an experimental approach. *Jyväskylän Studies in Biological and Environmental Science* 134: 1-38.

- Ruess, R. W. 1988. The interaction of defoliation and nutrient uptake in *Sporobolus kentrophyllus*, a short-grass species from the Serengeti Plains. *Oecologia* 77: 550-556.
- Ruess, R. W. & McNaughton, S. J. 1987. Grazing and the dynamics of nutrient and energy regulated microbial processes in the Serengeti grasslands. *Oikos* 49: 101-110.
- Sankaran, M. & Augustine, D. J. 2004. Large herbivores suppress decomposer abundance in a semiarid grazing ecosystem. *Ecology* 85: 1052-1061.
- Schimel, J. P. and Bennett, J. 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85: 591-602.
- Seastedt, T. R., Ramundo, R. A. & Hayes, D. C. 1988. Maximization of densities of soil animals by foliage herbivory: empirical evidence, graphical and conceptual models. *Oikos* 51: 243-248.
- Setälä, H. & Huhta, V. 1991. Soil fauna increase *Betula pendula* growth: Laboratory experiments with coniferous forest floor. *Ecology* 72: 665-671.
- Sohlenius, B. 1979. A carbon budget for nematodes, rotifers and tardigrades in a Swedish coniferous forest soil. *Holarctic Ecol.* 2: 30-40.
- Stanton, N. L. 1983. The effect of clipping and phytophagous nematodes on net primary production of blue grama, *Bouteloua gracilis*. *Oikos* 40: 249-257.
- Strauss, S. Y. & Agrawal, A. A. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.* 14: 179-185.
- Thornton, B. & Millard, P. 1996. Nitrogen uptake by grasses: changes induced by competing neighbour plants differing in frequency of defoliation. *Grass Forage Sci.* 51: 242-249.
- Todd, T. C. 1996. Effects of management practices on nematode community structure in tallgrass prairie. *Appl. Soil Ecol.* 3: 235-246.
- Todd, T. C., James, S. W. & Seastedt, T. R. 1992. Soil invertebrate and plant responses to mowing and carbofuran application in a North American tallgrass prairie. *Plant Soil* 144: 117-124.
- van Ruijven, J., De Deyn, G. B., Raaijmakers, C. E., Berendse, F. & van der Putten, W. H. 2005. Interactions between spatially separated herbivores indirectly alter plant diversity. *Ecol. Lett.* 8: 30-37.
- Verhoef, H. A. & Brussaard, L. 1990. Decomposition and nitrogen mineralization in natural and agroecosystems: the contribution of soil animals. *Biogeochemistry* 11: 175-211.
- Wall-Freckman, D. & Huang, S. P. 1998. Response of the soil nematode community in a shortgrass steppe to long-term and short-term grazing. *Appl. Soil Ecol.* 9: 39-44.
- Wardle, D. A. 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biol. Rev.* 67: 321-358.
- Wardle, D. A. 2002. *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. Princeton: Princeton university press.
- Wardle, D. A. & Barker, G. M. 1997. Competition and herbivory in establishing grassland communities: implications for plant biomass, species diversity and soil microbial activity. *Oikos* 80: 470-480.

- Wardle, D. A. & Yeates, G. W. 1993. The dual importance of competition and predation as regulatory forces in terrestrial ecosystems: evidence from decomposer food-webs. *Oecologia* 93: 303-306.
- Wardle, D. A., Yeates, G. W., Williamson, W. M., Bonner, K. I. & Barker, G. M. 2004. Linking aboveground and belowground communities: the indirect influence of aphid species identity and diversity on a three trophic level soil food web. *Oikos* 107: 283-294.
- Wilsey, B. J., Coleman, J. S. & McNaughton, S. J. 1997. Effects of elevated CO<sub>2</sub> and defoliation on grasses: a comparative ecosystem approach. *Ecol. Appl.* 7: 844-853.
- Yeates, G. W., Bongers, M., de Goede, R. G. M., Freckman, D. W. & Georgieva, S. S. 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. *J. Nematol.* 25: 315-331.
- Zar, J. H. 1999. *Biostatistical Analysis* (4. edition). New Jersey: Prentice Hall.