

Original Research

Microbial Abundance and Some of Their Physiological Activities in Soil Organic Horizon of Pine Forest Affected by Insect Herbivory

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Received: November 22, 2005

Accepted: June 2, 2006

Abstract

A phytophagous insect outbreak affects the ecosystem of a pine forest. In this study we present evidence that invertebrate activity affects not only trees, but also the soil microbial community in pine forest soil.

Numbers of actinobacteria and fungi increased significantly due to bigger supply of easily degradable organic matter in litterfall. High rates of respiration in soils of the affected forest sites have also been noted. However, high respiration intensities were observed for no longer than a year after herbivory outbreak. Moreover, herbivory did not cause major, long-term disruptions of nitrogen mineralization processes in the studied pine forest soil.

keywords: microbial activity, pine forest soil, herbivory, soil respiration, mineralization

Introduction

Outbreaks of defoliating insects are common phenomena in Polish coniferous forests. The majority of Polish forests consist of *Pinus sylvestris* L. stands. Their single-species nature is one of the main factors that enhance the susceptibility of these forests to herbivory. It may also cause the herbivory to have large-scale ecosystem impact.

The effects of herbivory on forest trees have been widely discussed [1, 2, 3]. Mattson and Addy [4] hypothesized that invertebrate herbivores are regulators of forest primary production, by altering matter quality and flow in forest ecosystems. Some authors have suggested that defoliation can also have adverse effects on forest ecosystems, such as: decrease of tree growth, increased tree mortality and water drainage [2, 3, 5]. Unfortunately, less

interest has been put into determining the impacts of herbivory on other organisms forming forest ecosystems, like soil microorganisms.

Herbivory outbreaks significantly influence soil chemistry [6]. During the outbreak the amount of organic matter reaching the forest floor increases. Chemistry of the organic matter is also altered. Besides elements of bark, twigs and leaves, organic matter contains a more easily degradable fraction of insect frass [7]. Organic matter, partly digested by herbivores, has lower C:N ratio than plant tissues. The amount of available nitrogen in forest soil can increase even threefold after a herbivory outbreak [8]. It is expected that changes in forest soil environment could influence activity of soil dwelling organisms (including microorganisms) and thus processes (e.g. mineralization) in the soil [9].

In this paper we examined the effect of the herbivory and its intensity on soil microorganisms' abundance and their physiological activity in pine forest soil. We determined that

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the numbers of three major groups of microorganisms in soil: heterotrophic bacteria, actinobacteria and heterotrophic fungi during a two-year period following phytophagous insects outbreak. We also examined microbial activity in selected biochemical soil processes at forest sites with different levels of foliage deterioration following insects' outbreak. This included measurement of soil respiration rate, ammonification potential and nitrification activity.

Materials and Methods

Study Area

The studied pine forest is located in Wymiarki forest inspectorate (51°31' N, 15°05' E) in Lubuskie Voivodship (southwestern Poland). The forest area was affected by phytophagous insects' outbreak in vegetative season 2001. The two main outbreak species were *Panolis flammea* (Den. et Schiff.) and *Dendrolimus pini* (L.). *Lymantria monacha* (L.) constituted less than 5% of phytophagous insects population. *Panolis flammea* (Den. et Schiff.) and *Lymantria monacha* (L.) caterpillars start feeding on pine needles in late spring and *Dendrolimus pini* (L.) caterpillars feed on needles from August until October. Forest canopy consisted of Scots pine (*Pinus sylvestris* (L.)). Undergrowth consisted of *Dicranum* sp. (Hedw.), *Hypnum jutlandicum* (Holmen & Warncke), *Calluna vulgaris* (L.) Hull and *Vaccinium vitis-idaea* (L.). Forest soils were categorized as podzolic.

Sampling

Mixed soil samples of F horizon were collected in late October 2001, April and October 2002, and April and November 2003. Four sample collection plots were established in forest sections, which differed in degree of defoliation by the insects' outbreak. Distance between individual forest sites was less than two kilometers.

Soil samples were collected at:

- 1) low herbivory site (section 135f- sprayed with Dimilin® insecticide in spring 2001, defoliation in 2001: 11-30%, 42 years old)
- 2) moderate herbivory site (section 113d- defoliated pine forest, defoliation in 2001: 31-60%, 40 years old)
- 3) high herbivory site (section 202b- heavily defoliated pine forest, defoliation in 2001: 61-90%, 45 years old)
- 4) control site (section 221a- pine forest stand not affected by herbivory, 45 years old).

Defoliation percentage was determined by Department of Forest Protection of Polish Forest Research Institute (data not published). At every forest section three mixed soil samples were collected (each one consisting of 5 individual, randomly collected subsamples) from the sample plot 100m x 100m. Before laboratory experiments, bark and twigs were removed manually from the samples and soil was sieved on Ø2mm sieve. Soils were kept at 5°C before conducting experiments (for a maximum of 2 weeks).

Soil Physical and Chemical Analysis

Dry weight of soil samples was determined after drying the samples (10g) at 105°C for 24 hours. Other chemical analyses of soil were conducted by Department of Forest Site Science at Forest Research Institute in Warsaw. Percentage of C and N content in soil dry mass was determined using LECO analyzer. Ammonium and nitrate contents in soil extracts (in 1M KCl) were determined colorimetrically. Values of pH were determined in 1M KCl (ratio soil: KCl was 1:5).

Numbers of Heterotrophic Bacteria

Quantification of culturable heterotrophic bacteria was performed in nutrient broth liquid medium (15g nutrient broth/1L H₂O) using the MPN (Most Probable Number) method. Dilutions were incubated at 30°C for 2 weeks. Suspension of 10g of moist soil samples in 95 mL of sterile 0.9% NaCl was used as 10⁻¹ dilution. The aliquot of this dilution (1mL) was then transferred to the first tubes (with 9mL of liquid medium) in MPN test. Subsequent dilutions (until 10⁻¹⁰ dilution) were made according to standard MPN test. For each soil 3 replicates, for determining Most Probable Numbers of heterotrophic bacteria were done.

Numbers of Actinobacteria

Determination of number of culturable actinobacteria (CFU- Colony Forming Units) was performed using plate count method on medium consisting of (per 1L H₂O): starch 10g; casein 0.3g; KNO₃ 2.0g; NaCl 2.0g; K₂HPO₄ 2.0 g; MgSO₄*7H₂O 0.05g; CaCO₃ 0.02g; FeSO₄*7H₂O 0.01g; agar 15g. Nystatin (100units/mL medium) and cycloheximide (50mg/mL medium) were added to prevent fungal growth [10]. The pH of the medium was adjusted to 5.5 before sterilization. Suspension of 10g of moist soil samples in 95 mL of sterile 0.9% NaCl was used as 10⁻¹ dilution. The aliquots (0.1mL) of appropriate dilutions (10⁻², 10⁻³, 10⁻⁴) were then plated on agar. For each dilution 3 replicates were done. Plates were incubated at 30°C for 10 days.

Numbers of Fungi

A number of Colony Forming Units (CFU) of culturable fungi was determined using plate count method on Rose Bengal Streptomycin Agar medium [11] consisting of (per 1L H₂O): glucose 10g; peptone 5g; K₂HPO₄ 1.0g; MgSO₄*7H₂O 0.5g; Rose Bengal 33mg; streptomycin 25 mg; agar 15g. The pH of the medium was adjusted to 5.5 before sterilization. Suspension of 10g of moist soil samples in 95 mL of sterile 0.9% NaCl was used as 10⁻¹ dilution. The aliquots (0.1mL) of appropriate dilutions (10⁻², 10⁻³, 10⁻⁴) were then plated on agar. For each dilution 3 replicates were done. Plates were incubated at 30°C for 1 week.

Soil Respiration in Pine Forests

Soil respiration measurements were performed at 25°C using IRGA (Infrared Gas Analyser) [12]. Soil sample (5g wet weight) was closed in an air-tight container (of a known cubature) and the CO₂ evolution was measured. Three replicates were done for each soil. Intensity of soil respiration was expressed in µg CO₂/ g dry soil weight/ hour.

Potential Ammonification Activity in Pine Forest Soils

Determination of potential ammonification activity in pine forest soil samples was performed using a modification of the Alef and Kleiner method [13]. Soil samples (30g) were incubated for 24 hours with the addition of arginine (2mg/g of dry soil weight) at room temperature. Three replicates were done for each soil. Soil solution was prepared for the measurement of NH₄-N concentration in soil at the beginning and the end of incubation. Soil (10g wet weight) was shaken with 100mL of 1M KCl solution for 1.5 hours at 125rpm. Soil solution was then filtered on Whatman 2.0 filter paper. The filtrate was analyzed spectrophotometrically (on Spectronic® 20 Genesys™ spectrophotometer, Spectronic Instruments, USA) for the presence of NH₄-N using Spectroquant® 1.14752 kit (Merck). Net potential ammonification activity was expressed in µg NH₄-N/ g dry soil weight/day.

Nitrification Activity in Pine Forest Soils

Determination of nitrification activity in pine forest soil was performed with soil incubation method [14]. Three replicates were done for each soil. Water content was checked daily and adjusted to the start value. Soil samples (30g) were incubated for 6 weeks in the dark at 22°C. Measurement of NO₃-N content at the beginning and the end of incubation was done spectrophotometrically in soil solution prepared as in the potential ammonification experiment. The method used to determine presence of nitrate ions involved electrophilic aromatic substitution (nitration) between nitronium and salicylate. Measurements were done using Spectronic® 20 Genesys™ spectrophotometer (Spectronic Instruments, USA). Net nitrification activity was expressed in µg NO₃-N/ g dry soil weight/week.

Statistical Analysis

To determine the effect of sampling site on microbial activity a one-way ANOVA test was used. We also determined the effect of sampling date on microbial activities in soil at individual sites using the above-mentioned test. Homogeneity of the variance of the samples was checked

with Levene's statistics prior to ANOVA test. Two-way ANOVA was used to analyze the effects of sampling site and sampling date (within years 2002 and 2003) and the interaction of these factors on microbial abundance, soil respiration, ammonification potential and nitrification activity. Correlations between soil microorganisms' numbers and their activities and soil microorganisms' numbers and chemical properties were determined by Pearson correlation coefficient. For statistical analysis SPSS 12.0 PL for Windows program was used.

Results

Chemical Properties of Studied Soils

Results of chemical analysis of soil FH horizon in fresh pine forest in Wymiarki inspectorate are presented in Table 1. Soil of the low and moderate herbivory sites (defoliation <60%) showed higher nitrogen content (2-fold) in sampling following the outbreak than in the samplings in the next two years. The nitrogen contents were 0.66±0.13% and 0.76±0.18% of dry mass for low and moderate herbivory sites, respectively. On above-mentioned sites contents of ammonium-nitrogen were higher in soil in October 2001 sampling than in April 2002. On the heavy herbivory site nitrogen content remained low throughout first year after defoliation. Slight increase in nitrogen content in soil was noted for heavy herbivory site in year 2003 samplings. Ammonium content in soil of outbreak affected sites was significantly higher in sampling following outbreak (autumn 2001) than in spring sampling in 2002. Nitrates content of all soil samples remained low throughout samplings in 2001-03.

Forest soils from low and moderate herbivory sites showed higher organic C content than on the heavy herbivory site in autumn sampling in 2001. The carbon content was 14.03±3.85%, 17.70±2.52% and 9.21±1.11% of dry mass for low, moderate and heavy herbivory sites, respectively. Carbon content in soil at low and moderate herbivory sites remained stable throughout 2001-03 samplings. Organic C content in soil at heavy herbivory site increased the year after the leaf-eating insect outbreak to 14.36±1.01% of dry mass. Control site showed the two-fold increase of organic C content in the year 2003 samplings (17.30±2.86% of dry mass) in comparison with previous samplings (8.45±1.83% and 8.27±2.07% of dry mass for spring 2002 and autumn 2002 sampling, respectively).

Microbial Abundance in Soils of Pine Forests Affected by Phytophagous Insects' Outbreaks

Results of two-way ANOVA showed no significant effect of sampling site, sampling date and interaction of both factors on culturable heterotrophic bacteria numbers in the years 2002 and 2003 (Table 2). During the sampling

Table 1. Chemical properties of soil F horizon of the pine forest sites affected by herbivory outbreak.

Site	Date	pH (in KCl)	%C	%N	C:N	NH ₄ -N (mg/100g soil)	NO ₃ -N (mg/100g soil)
low herbivory	X 2001	2.96 (0.11)	14.03 (3.85)	0.66 (0.13)	21.00 (1.70)	3.40 (0.99)	0.70 (0.70)
	IV 2002	2.96 (0.12)	11.43 (4.70)	0.34 (0.12)	33.73 (4.92)	1.68 (0.56)	0.98 (0.74)
	X 2002	2.81 (0.07)	11.36 (0.56)	0.36 (0.01)	31.20 (1.92)	3.33 (0.72)	0.93 (0.42)
	IV 2003	2.88 (0.03)	18.86 (7.46)	0.49 (0.05)	37.10 (11.46)	4.31 (0.80)	3.12 (2.02)
	XI 2003	2.94 (0.07)	24.26 (2.81)	0.73 (0.12)	33.23 (1.75)	3.03 (0.74)	1.19 (1.04)
moderate herbivory	X 2001	2.96 (0.05)	17.70 (2.52)	0.76 (0.18)	23.83 (2.66)	5.46 (2.38)	0.84 (0.00)
	IV 2002	2.95 (0.06)	13.50 (1.63)	0.38 (0.08)	35.33 (3.11)	1.96 (0.77)	0.42 (0.24)
	X 2002	2.80 (0.01)	12.93 (2.21)	0.38 (0.01)	33.90 (5.66)	4.08 (0.49)	4.03 (2.70)
	IV 2003	2.93 (0.01)	18.66 (4.85)	0.47 (0.12)	40.23 (8.04)	6.44 (1.81)	3.54 (1.57)
	XI 2003	2.91 (0.06)	13.40 (4.95)	0.45 (0.15)	29.50 (1.23)	3.08 (0.25)	1.33 (1.44)
high herbivory	X 2001	3.03 (0.06)	9.21 (1.11)	0.34 (0.14)	29.17 (8.38)	3.54 (0.77)	1.07 (0.40)
	IV 2002	3.03 (0.01)	8.59 (2.46)	0.24 (0.05)	37.26 (2.88)	2.05 (0.40)	0.42 (0.28)
	X 2002	2.83 (0.02)	14.36 (1.01)	0.48 (0.04)	29.50 (0.78)	4.50 (0.32)	0.81 (0.47)
	IV 2003	2.98 (0.02)	15.63 (3.36)	0.51 (0.11)	30.66 (0.37)	5.50 (0.21)	1.75 (1.58)
	XI 2003	2.98 (0.03)	20.33 (2.95)	0.65 (0.06)	30.96 (2.80)	2.63 (0.35)	4.76 (3.86)
control	X 2001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	IV 2002	3.01 (0.11)	8.45 (1.83)	0.23 (0.05)	36.16 (1.87)	0.98 (0.28)	0.14 (0.14)
	X 2002	2.96 (0.07)	8.27 (2.07)	0.48 (0.05)	29.67 (2.67)	3.96 (0.56)	2.21 (1.29)
	IV 2003	2.99 (0.04)	17.30 (2.86)	0.53 (0.06)	32.70 (5.26)	4.50 (1.27)	3.33 (1.56)
	XI 2003	3.00 (0.01)	15.00 (2.97)	0.48 (0.09)	31.23 (5.40)	2.40 (0.26)	4.43 (4.10)

low herbivory- section 135f, pine forest sprayed with Dimilin insecticide (2001), 42 years old, defoliation 11-30% (2001); moderate herbivory- section 113d, pine forest, 40 years old, defoliation 30-60% (2001); high herbivory- section 202b, pine forest, 45 years old, defoliation 60-90% (2001); control- section 221a, pine forest not affected by herbivory, 45 yrs old; n.d- not determined; Standard deviation is shown in brackets

in autumn 2001, after the occurrence of insect outbreak, the abundance of culturable heterotrophic bacteria was significantly higher in soil of the moderate herbivory site compared to low herbivory site (sprayed with insecticide) (Fig. 1). There has been no significant change in heterotrophic bacteria numbers at the individual site during the course of the two years of study (Fig. 2). This was true for all forest sites studied, regardless of herbivory outbreak history. Culturable heterotrophic bacteria numbers were not significantly correlated with soil organic C content, soil organic N content and with C:N ratio.

Numbers of culturable actinobacteria Colony Forming Units (CFU) in the soils of herbivory-affected sites were significantly higher in 2001 sampling compared to later sampling dates (Fig. 2). There was no significant effect of sampling site, sampling date and combination of both factors on culturable actinobacteria numbers in the years 2002 and 2003 (Table 2). On these sampling dates numbers of actinobacteria remained stable. Culturable actinobacteria abundances correlated positively

with soil organic N content at the moderate herbivory site ($r=0.686$, $P=0.01$). No significant correlation between soil organic C and numbers of culturable actinobacteria was noted. Significant negative correlations were noted between actinobacteria numbers and soil C:N ratio for the low and moderate herbivory sites. The Pearson's correlation coefficients were $r=-0.712$ ($P=0.01$) and $r=-0.666$ ($P=0.01$) for the low and moderate herbivory sites, respectively.

Similar pattern of changes in abundances, as the one of actinobacteria, could be seen for numbers of heterotrophic fungi from pine forest soils studied (Fig. 2). The sampling in the year 2001 showed high numbers of fungi in the soils of sites affected by the outbreak. A sharp decrease in numbers of heterotrophic fungi was seen in samples taken during 2002 and 2003. Two-way analysis of variance (two-way ANOVA) showed that sampling site, sampling date and combination of both factors had a significant effect on numbers of heterotrophic fungi in the years 2002 and 2003 (Table 2). At the control site numbers

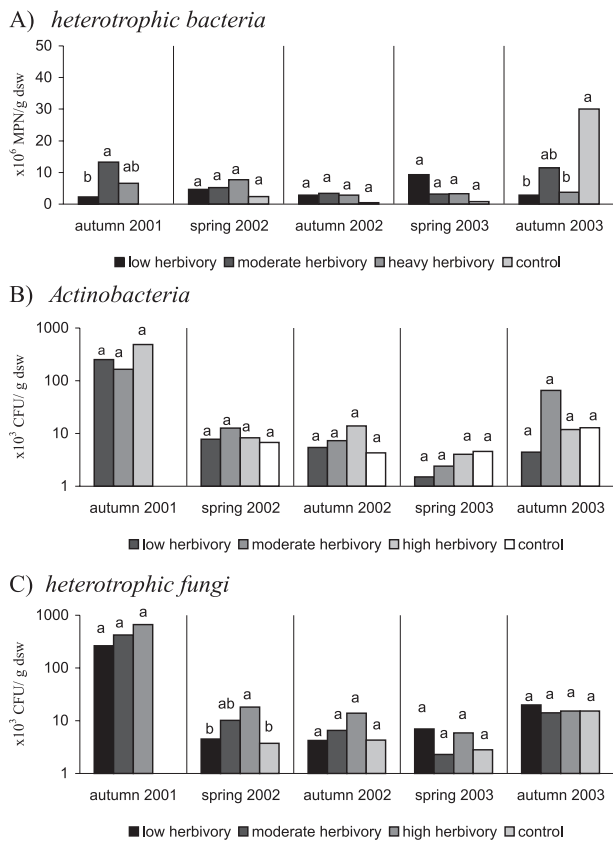


Fig. 1. Abundance of three main groups of microorganisms in soil of pine forest affected by herbivory.

low herbivory- section 135f, pine forest sprayed with Dimilin insecticide (2001), 42 years old, defoliation 11-30% (2001), **moderate herbivory**- section 113d, pine forest, 40 years old, defoliation 30-60% (2001), **high herbivory**- section 202b, pine forest, 45 years old, defoliation 60-90% (2001), **control**- section 221a, pine forest not affected by herbivory, 45 years old, **dsw**-dry soil weight (determined after drying at 105°C).

Numbers of microorganisms represented by bars marked with the same letter (within the same sampling date) do not differ significantly from each other ($P=0.05$).

of fungi remained stable. The only exception was sampling in autumn 2003 when the numbers of fungi at this site increased fivefold compared to previous samplings. Numbers of heterotrophic fungi correlated positively with soil organic N content at the moderate herbivory site ($r=0.688$, $P=0.01$). Significant negative correlations between numbers of heterotrophic fungi and soil C:N ratio were noted. The Pearson's correlation coefficients were $r=-0.692$ ($P=0.01$) and $r=-0.608$ ($P=0.01$) for the low and moderate herbivory sites, respectively.

Microbial Activity in Soils of Pine Forests Affected by Outbreaks of Phytophagous Insects

Three microbiological processes in forest soil were studied. Firstly, we determined the intensity of soil res-

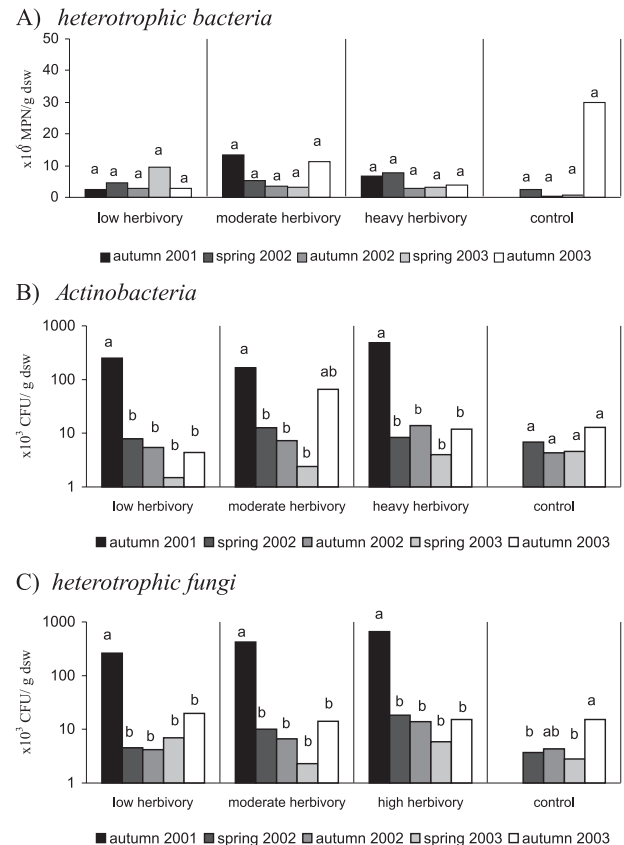


Fig. 2. Abundance of three main groups of microorganisms in soil of pine forest affected by herbivory.

low herbivory- section 135f, pine forest sprayed with Dimilin insecticide (2001), 42 years old, defoliation 11-30% (2001), **moderate herbivory**- section 113d, pine forest, 40 years old, defoliation 30-60% (2001), **high herbivory**- section 202b, pine forest, 45 years old, defoliation 60-90% (2001), **control**- section 221a, pine forest not affected by herbivory, 45 years old, **dsw**-dry soil weight (determined after drying at 105°C).

Numbers of microorganisms represented by bars marked with the same letter (within the same sampling site) do not differ significantly from each other ($P=0.05$).

piration, which would show a general picture of activity of microflora. Soil samples from autumn 2001 (samples from forest site affected by insects' outbreak), collected in late October, showed high soil respiration intensity (Fig. 3). Carbon dioxide evolution was higher in the soils of forest sites with 30-60% defoliation (moderate herbivory site) and at the site which was treated with insecticide (low herbivory site) than at the forest site with higher defoliation (heavy herbivory site). Measured soil respiration intensities were 123.16 ± 11.80 , 120.27 ± 20.07 , and 77.72 ± 21.60 $\mu\text{g CO}_2/\text{g dry weight}/\text{hour}$ for low, moderate and heavy herbivory sites, respectively. Herbivory-affected sites showed decrease in soil respiration intensity on sampling dates in 2002 and spring 2003 compared to 2001 (Fig. 4). Soil respiration at the control site in 2002 was lower than at sites affected by phytophagous insect outbreaks in the previous year. During 2003 samplings the

Table 2. Analyses of variance (two-way ANOVA) to estimate the effects of sampling site and sampling date on microbial abundance, soil respiration, ammonification potential and nitrification activity in soils of pine forests.

	heterotrophic bacteria			actinobacteria			heterotrophic fungi		
	df	F	P	df	F	P	df	F	P
site (S)	3	1.270	0.301	3	2.079	0.123	3	8.271	<0.001
date (D)	3	1.449	0.247	3	2.881	0.51	3	24.330	<0.001
S x D	9	0.578	0.805	9	1.479	0.198	9	3.580	0.004
	soil respiration			ammonification potential			nitrification activity		
	df	F	P	df	F	P	df	F	P
site (S)	3	75.773	<0.001	3	0.668	0.578	3	4.006	0.016
date (D)	3	131.438	<0.001	3	10.077	<0.001	3	18.339	<0.001
S x D	9	33.741	<0.001	9	0.212	0.971	9	2.570	0.024

For analysis of variance (two-way ANOVA) only sampling dates within years 2002 and 2003 were used.

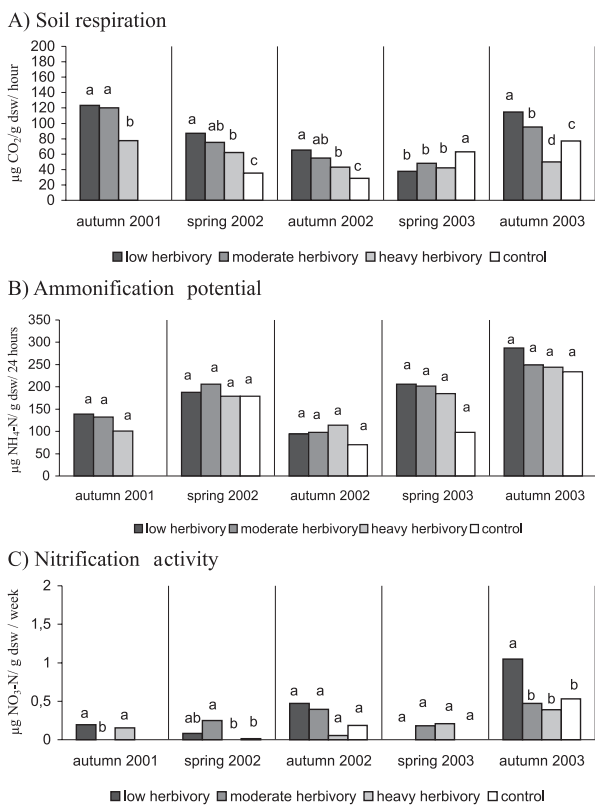


Fig. 3. Soil respiration, ammonification potential and nitrification activity in soils of pine forests affected by herbivory.

low herbivory- section 135f, pine forest sprayed with Dimilin insecticide (2001), 42 years old, defoliation 11-30% (2001), **moderate herbivory**- section 113d, pine forest, 40 years old, defoliation 30-60% (2001), **high herbivory**- section 202b, pine forest, 45 years old, defoliation 60-90% (2001), **control**- section 221a, pine forest not affected by herbivory, 45 years old, **dsw**-dry soil weight (determined after drying at 105°C).

Values represented by bars marked with the same letter (within the same sampling date) do not differ significantly from each other ($P=0.05$).

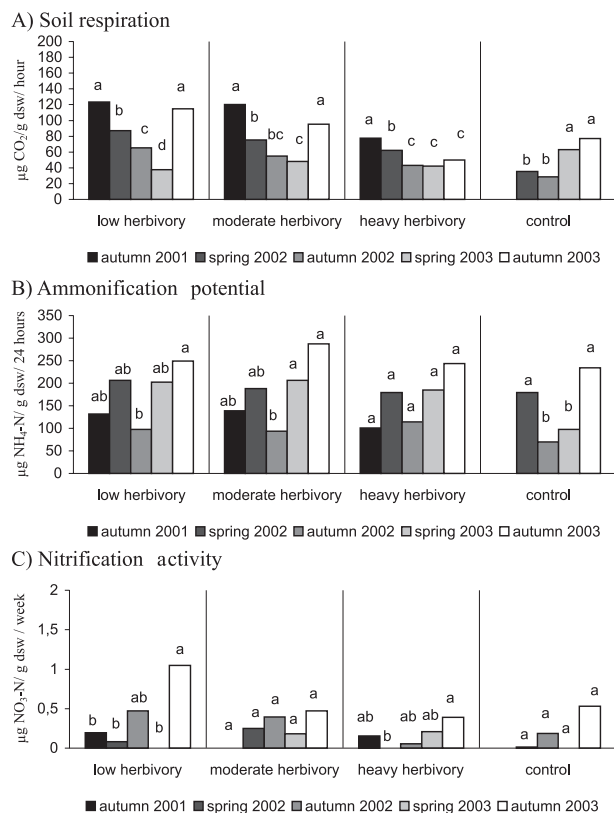


Fig. 4. Soil respiration, ammonification potential and nitrification activity in soils of pine forests affected by herbivory.

low herbivory- section 135f, pine forest sprayed with Dimilin insecticide (2001), 42 years old, defoliation 11-30% (2001), **moderate herbivory**- section 113d, pine forest, 40 years old, defoliation 30-60% (2001), **high herbivory**- section 202b, pine forest, 45 years old, defoliation 60-90% (2001), **control**- section 221a, pine forest not affected by herbivory, 45 years old, **dsw**-dry soil weight (determined after drying at 105°C).

Values represented by bars marked with the same letter (within the same sampling site) do not differ significantly from each other ($P=0.05$).

increase in soil respiration was observed in the soil at this site. Two-way ANOVA analysis performed for sampling dates in the years 2002 and 2003 showed that sampling site, sampling date and combination of both factors had significantly affected soil respiration (Table 2). Soils of forests affected by herbivory in 2001 showed a significant increase in soil respiration on the last date of sampling in November 2003. Respiration intensities at the low and moderate herbivory sites were higher than the ones of the control site in autumn 2003.

Soil respiration was positively correlated with soil organic N content at the low herbivory site ($r=0.568$, $P=0.05$), the moderate herbivory site ($r=0.656$, $P=0.01$) and the control site ($r=0.749$, $P=0.01$). Negative correlation between soil respiration and soil C:N ratio was noted for the low herbivory and the moderate herbivory sites. The Pearson's correlation coefficients were $r=-0.470$ ($P=0.05$) and $r=-0.735$ ($P=0.01$) for the low and moderate herbivory sites, respectively. Statistical analysis showed also positive correlation between numbers of culturable actinobacteria and fungi in soil and respiration rates for all sampling sites. Pearson correlation coefficient for correlation between actinobacteria numbers and respiration rate was: $r=0.564$ ($P=0.05$) for the low herbivory site, $r=0.641$ ($P=0.01$) for the moderate herbivory site, $r=0.507$ ($P=0.05$) for the heavy herbivory site and $r=0.617$ ($P=0.05$) for the control site. Pearson's correlation coefficient between fungi numbers and respiration rate was: $r=0.588$ ($P=0.05$) for the low herbivory site, $r=0.702$ ($P=0.01$) for the moderate herbivory site, $r=0.614$ ($P=0.05$) for the heavy herbivory site and $r=0.667$ ($P=0.05$) for the control site. Culturable heterotrophic bacteria numbers correlated positively with respiration rates only at the moderate herbivory site ($r=0.623$, $P=0.05$). For other sites no significant positive correlation between heterotrophic bacteria numbers and respiration rate was observed.

Potential ammonification was assessed to determine if the changes in environmental factors in pine forest soil, caused by herbivory, did affect the rate of microbial mineralization potential of studied soils (Fig. 3). In autumn 2001 there was no significant difference between the potential ammonification activity in soils differently affected by herbivory. Ammonification potential was 132.56 ± 59.24 , 139.18 ± 43.45 , and 101.27 ± 45.05 $\mu\text{g NH}_4\text{-N/g dry weight/ 24 hours}$ for the low, moderate and heavy herbivory sites, respectively. On later sampling dates no significant difference in ammonification potential among studied forest sites was detected between the herbivory affected and unaffected sites.

Significant effect of sampling date on ammonification potential was noted (Table 2). When the effect of sampling date on ammonification potential at individual forest sites was studied, it was shown that there was no significant difference in ammonification potential in the soil of the heavy herbivory site (Fig. 4). However, at the low herbivory site soil samples from autumn 2002 showed significantly lower ammonification potential compared to values measured for samples from autumn 2003. Ammo-

nification potential, measured at this site in autumn 2002 was not however, significantly different from potentials measured remaining sampling dates (autumn 2001 and spring 2002 and 2003). Ammonification potential in soil of the moderate herbivory site and of the control site showed the same pattern of sampling date influence on the process. Potential has decreased in autumn 2002 sampling compared with spring 2002 at both above-mentioned sites. In 2003 potential for ammonification increased at both moderate herbivory and control sites compared with previous sampling dates.

Nitrification activity of a studied pine forest remained low regardless of phytophagous insects' outbreak history of the sites (Fig. 3). In spring 2002 sampling highest nitrification activity among all studied sites was observed at moderate herbivory site (0.25 ± 0.05 $\mu\text{g NO}_3\text{-N/g dry weight/ week}$). Nitrification activity at the moderate herbivory site (defoliation 30-60% in 2001) has shown no temporal differences (Fig. 4). The highest nitrification activity, which has been observed during 3 years of sampling, was 1 $\mu\text{g NO}_3\text{-N/g dry weight/week}$ in autumn 2003 at the low herbivory site (sprayed with Dimilin insecticide in 2001). On that sampling date the highest nitrification for control site was also observed (0.53 ± 0.22 $\mu\text{g NO}_3\text{-N/g dry weight/ week}$). Nitrification at the heavy herbivory site remained low throughout experimental period and never exceeded 0.4 $\mu\text{g NO}_3\text{-N/g dry weight/ week}$.

Discussion

Herbivory outbreaks in forested ecosystems cause major disruption to ecosystems. According to some authors these effects include loss of nitrogen, through nitrate leaching from soil [15, 16], tree mortality and water drainage [1, 2, 3]. The most extensively studied, however, was the response of the trees to foliar herbivory in outbreak situations. In this paper we examined the microbial community numbers and selected activity associated with environmental change in the soil of pine forests affected by herbivory.

Chemical composition of studied soils was significantly affected by phytophagous insect outbreaks. Carbon content, determined immediately after the phytophagous insects' outbreak, in the soils of the low and moderate herbivory sites were higher compared to the high herbivory site. Higher carbon contents were also observed by other authors in the soils of coniferous forests following phytophagous insect outbreaks [8, 17]. Higher amounts of organic matter reaching soils at the low and moderate herbivory sites can be attributed to fact, that on the high herbivory site caterpillars' numbers were higher, and due to their feeding less litterfall reached forest soil. At the low and moderate herbivory sites, apart from insects frass, partly digested needles reached the forest floor, which caused organic matter content to increase compared with the heavy herbivory site. "Sloppiness" in caterpillars' eat-

ing habits also caused an increase in nitrogen content in soils at the low and moderate herbivory sites. Nitrogen content at the low and moderate herbivory sites were almost twofold higher than at the high herbivory site. Dziadowiec and Plichta [8] also reported a threefold increase in nitrogen content of soil during outbreaks. In our studies we observed a 50% decrease of nitrogen content in the soil of low and moderate herbivory sites during sampling in 2002 (a year after the phytophagous insects outbreak). Dziadowiec and Plichta reported decreases in nitrogen content in soil, one year after outbreak, to levels lower than those observed before the outbreak [8].

To study microbial abundance in pine forest soils, viable plate count and Most Probable Number (MPN) methods were used. Authors are aware of the restrictions of these methods when used for quantification of microorganisms from environmental samples. Plate counts of bacteria from environment such as soil or water are generally lower than direct counts. It is estimated that less than 1% of bacteria are culturable [18]. These methods may also be selective for certain fast-growing organisms [19]. However, these methods are still used if high biodiversity of culturable microorganisms is expected in the studied environment.

Previous results of bacterial biodiversity studies in coniferous forest soils showed, using molecular biology methods, that *Proteobacteria*, *Actinobacteria* and *Firmicutes* may form the majority of bacterial communities in these soils [20, 21, 22]. These phylogenetic groups are well studied and about 70 to 80% of their known members are culturable [23]. Therefore, authors decided to use plate count and Most Probable Number (MPN) methods to monitor the numbers of culturable microbial communities in pine forest soil as an indication of possible overall changes in abundance.

Microbial abundance studies in the forest soils in Wymiarki inspectorate showed that numbers of culturable heterotrophic bacteria (CFU/g d.s.w. soil) did not change significantly due to herbivory in the pine forest. On a contrary culturable actinobacteria and heterotrophic fungi numbers were higher in the herbivory outbreak season than in later samplings. This may be the result of increased quality of litter that reaches the forest floor (as shown by correlation results between culturable actinobacteria and heterotrophic fungi and soil C:N ratio for the low and moderate herbivory sites). Actinobacteria are known to be active decomposers of humate- and chitin-containing organic matter in forest soil [24]. Heterotrophic fungi's preferable carbon source is easily degradable organic matter (e.g. insects' caterpillars frass). Both microbial groups thrive better in the soils of the defoliated forest sites in the year of the phytophagous insects' outbreak compared with later samplings. High numbers of culturable actinobacteria and fungi was also noted for the moderate herbivory site and the control site in autumn 2003. Autumn sampling in 2003 was carried out in November after the litterfall. Large amounts of organic matter reached the forest floor, but in 2003 litter "quality" was

lower compared to autumn 2001. Litterfall in 2003 lacks the component of insects' frass, whose chemical composition lowered the C:N ratio, enabling faster decomposition of organic matter. High numbers of culturable actinobacteria and fungi at these forest sites may also be associated with transport of microorganisms from trees' phylloplane to forest floor. Researchers studying heterotrophic bacteria on phytophagous insects that infested Norway spruce trees found that herbivory significantly increases abundance of aerobic heterotrophs (e.g. bacteria and fungi) in phylloplane of these trees [25]. On the contrary, in studies on the effect of pinyon pine stress caused by *Dioryctria albovittella* on bacterial heterotrophs, authors showed no significant increase in numbers of bacterial heterotrophs, actinomycetes and fungi in rhizospheres of resistant, susceptible and sprayed with insecticide trees [26]. It has to be noted, though, that *D. albovittella* is a stem-boring moth in contrast to mesophyll-feeding insects investigated in our study and trees studied were affected by chronic herbivory for at least 18 years.

Determination of the soil respiration rates in the studied forest soils showed that herbivory had positive effect on soil respiration intensity. Respiration intensity was significantly higher in autumn 2001 sampling, which took place after the herbivory outbreak, than on later sampling dates in 2002 and spring 2003. Results show also no adverse impact of insecticide treatment on soil respiration rates at the low herbivory site. Dimilin insecticide (active ingredient diflubenzuron) treatment is unlikely to cause effect on forest soil bacteria and fungi due to the low dosage used (around 75 g of active ingredient/ ha) [27]. Furthermore, in forest management practise insecticide is only used once a year. It has been shown that Dimilin treatment had no effect on the rate of organic matter decomposition in forest soil [28]. The effect of herbivory on soil respiration was also observed in hardwood forest [29]. In this study, though, authors did not observe significant change of respiration rate in treatment of forest floor with insects' frass additions. They note, however, that the amount of frass added was to mimic the situation in case of endemic insect densities, not an outbreak situation. Lovett and Ruesink [30] reported significant increase in soil respiration (to more than 2000 $\mu\text{g C/g dry weight/ 24 hours}$) in deciduous forest following the addition of large amounts of phytophagous insects' frass. In their study, significantly higher intensity of soil respiration was observed in frass treatments, compared with control soil, up to 30 days after frass addition.

In autumn 2001 sampling soil respiration rate on the heavy herbivory site was significantly lower than on the low and moderate herbivory sites, despite no significant differences in abundance of culturable heterotrophic bacteria, actinobacteria and fungi. This could be partly explained by soil chemistry comparison discussed earlier in this article. Chemical characteristics of soil organic matter might have slowed down degradation processes on the heavy herbivory site. This site showed also lowest respiration rate in November 2003. This can be explained by

lower litterfall (and thus lower organic matter transport to forest floor) due to the fact that trees on this site lost a substantial part of their foliage in 2001. Needles, which regenerated on the trees at this site were not subject to autumn litter fall in 2003.

It is worth mentioning that in all defoliated forest sites respiration activity increased in the November 2003 sampling. This can be explained by the addition of freshly fallen needles to the forest floor (due to autumn litterfall) and thus supplying the microbial community with more organic substrates for respiration.

Results of statistical correlation analysis between respiration rates and numbers of main microbial groups in pine forest soil suggest that during herbivory outbreak, actinobacteria and fungi are more active microbial groups. Cullings *et al.* suggested that even small changes in fungal biodiversity, occurring during defoliation of pine forest, may increase physiological capabilities of the overall fungal community in soil [31].

Ammonification potential in the soils of the control, low herbivory and moderate herbivory sites has shown statistically significant seasonal differences. Generally, ammonification potential was higher during spring samplings than autumn samplings in October. However, when ammonification potential of the soils at the sites, differing in herbivory history, were compared, no significant differences were found. High ammonification potential on the control site and the moderate herbivory site in autumn 2003 may be due to the high activity of ammonifying microorganisms after litterfall. As we studied net ammonification potential (difference between gross ammonification and ammonium consumption rate), lower amounts of ammonium liberated into soil in samples from 2001 compared to November 2003 may be due to higher incorporation or turnover rate of ammonium-N by more active microorganisms' cells in autumn 2001.

The pine forest soils studied exhibited low nitrification potential during the whole experiment. Amounts of nitrate were below 1 $\mu\text{g NO}_3^-$ -N/g dry weight soil/week. These result allows us to categorize studied pine forest as a so-called "non-nitrifying" ecosystem. Other authors have reported no net nitrification in different coniferous forests due to unfavourable environmental conditions [32, 33]. Maartikainen reported net nitrification (difference between gross nitrification and nitrate consumption rate) levels in northern coniferous forests to be below 6 $\mu\text{g NO}_3^-$ -N/g dry weight soil/6 weeks even in fertilized forest soil. It is unlikely then to face nitrogen losses through nitrate leaching in this ecosystem. Nitrogen losses through leaching are considered one of the adverse herbivory effects on forest ecosystems [15]. It was also shown though, by other experimental results, that defoliation causes reallocation of nitrogen resources in ecosystems rather than extreme losses of this element [34].

A few conclusions can be drawn from our results:

1. Phytophagous insect outbreaks affect soil microbial communities in pine forest soil. Numbers of culturable

actinobacteria and fungi increase significantly due to the supply of easily degradable organic matter in litterfall (with lower C:N ratio). Numbers of culturable heterotrophic bacteria were not significantly affected by herbivory outbreak.

2. Herbivory outbreaks increase overall soil respiration rates in soils of affected forest sites. This could be due to accelerated soil organic matter degradation by actinobacteria and fungi. Higher respiration rates were observed for no longer than a year after herbivory outbreak. On the heavy herbivory site (60-90% defoliation) positive effects of accelerated organic matter degradation were lessened by overall decrease in activity of organisms in heavily disrupted ecosystem. It has been shown that soil respiration rate increases, to levels observed during herbivory, also after a fresh litterfall (shown in sampling of November 2003).
3. Mineralization processes (ammonification and nitrification) are not likely to cause nitrogen losses in studied pine forest ecosystems. This is due to quick immobilization of released inorganic nitrogen by soil microorganisms and trees.

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