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The Effect of Grazing on Soil Microbial Biomass and Community on Alpine Pastures

By

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Key words: Soil microbial biomass, Biolog®, microbial community, metabolic quotient, organic matter, land use change, pasture.

Summary

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Within in a multidisciplinary project the effect of termination of cattle grazing on grassland and forest on soil microbial properties was investigated. The changes of microbial biomass (C_{mic}), basal respiration and the ecophysiological parameters (metabolic quotient and the C_{mic}:C_{org} ratio) were small. Mainly in the dry summer of 1993 an increase of basal respiration, microbial biomass and the C_{mic}:C_{org} ratio was observed for the fenced in sites. The effects were not observed in the moist summer of 1995. For the pasture site, a substrate utilization assay employing 95 different C sources (Biolog®) indicated some changes in the functional abilities of the bacterial communities after 9 years of protecting the site from grazing. The changes were attributed to a change of litter quality.

Introduction

The effects of changing land-use on the functioning of ecosystems have frequently been investigated. Considerable changes in ecosystem functioning can be expected through animal grazing. However, the observed effects, especially regarding the plant's compensation of tissue loss through grazing, are often contradictory (KIEFT 1994). HOLLAND & DETLING 1990 and PANDEY & SINGH 1992 found that aboveground grazing by animals drastically reduced belowground plant biomass. In contrast to that, RUESS & MCNAUGHTON 1987 reported that intensively grazed grassland had higher organic and microbial biomass carbon (C_{mic}) contents than control grassland without grazing. In any case, grazing may affect the organic

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matter status of soils which may increase its susceptibility to erosion. This may be of particular importance on steep alpine slopes, or in treeline forests where the climatic stress is strong and the forest has an important protective function.

Intimately linked with the organic matter status are the soil microorganisms, frequently referred to as microbial biomass. Microorganisms are the main mediator of organic matter turnover, and the C_{mic} fraction of organic carbon (C_{org}) is considered a very sensitive parameter for changes in the organic matter status (POWLSON & al. 1987). An increased C_{mic}/C_{org} ratio is found in soils that are in a state of C accumulation (INSAM & DOMSCH 1988), which may be the case on revegetation sites or on sites where a monoculture is replaced by crop rotation (ANDERSON & DOMSCH 1989). Basal respiration of soils indicates the endogenous availability of C for microorganisms, and the specific respiration of the biomass (metabolic quotient qCO_2) is known to be a good indicator for ecosystem disturbances (INSAM & al. 1996b).

Microbial substrate utilization is one of the functions that might be affected by ecosystem disturbance (BURKHARDT & al. 1993). GARLAND & MILLS 1991 proposed the use of Biolog® microtiter plates for assessing the substrate utilization by microbial communities. This technique has successfully been employed for determining differences between soils, studying microbial diversity in the rhizospheres of different tree species, or for characterizing microbial communities during decomposition processes (GRAYSTON & al. 1994, INSAM & al. 1996a, ZAK & al. 1994). If termination of grazing causes changes in the plant community, it is possible that through changes in organic matter input quality the functional abilities of the microbial community change, without bulk biomass or turnover processes being affected.

The present study investigates the effects of terminating grazing on formerly grazed alpine pasture and an adjacent open-spaced spruce forest. The parameters determined were C_{org}, C_{mic}, basal respiration, the ecophysiological quotients (C_{mic}/C_{org} ratio, qCO_2) as well as the functional abilities (diversity) of the microbial communities. The aim was to obtain basic information for long-term monitoring as well as possible short-term effects.

Materials and Methods

Sites

The studies were perfomed on the Schulterberg (near Achenkirch, Austria, 1600 m a.s.l.), where 15*15 m areas on a pasture and in a forest were fenced in and thus protected from cattle and game grazing (the experimental setup and description of the sites are given by SOBOTIK & POPPELBAUM 1995).

Site 1: Pasture, fenced in since 1986 (cattle and game proof)
Site 2: Pasture, cattle and wildlife grazing (control pasture)
Site 3: Open spaced spruce forest, fenced in since 1992 (cattle fence, but not game proof)
Site 4: Open spaced spruce forest, cattle and wildlife grazing (control forest)

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The soils (rendzinas, see ENGLISCH 1992) were sampled with a 7 cm diameter corer to a depth of 20 cm on July 14, 1992, on July 7 and September 22, 1993, and on July 7 and October 2, 1995. In the first year, four replicate samples were taken from each plot. Due to the high variability of the data, six samples were taken at each sampling date in all subsequent years. For the analyses, the samples were divided in two subsamples (0-10 and 10-20 cm). In the laboratory, the samples were sieved (2 mm) and their water content was adjusted to approximately -300 kP tension.

Analytical

Soil basal respiration was determined according to PARKINSON & al. 1978. Basal respiration is the release of CO_2 of a sieved soil without the addition of C or nutrients, given in µg CO_2 g^{-1} soil h^{-1}. Basal respiration is thus a measure of respiratory activity depending on the endogenous energy sources in the soil, relating it closely to the carbon cycle. Microbial biomass was determined with substrate induced respiration (SIR) (ANDERSON & DOMSCH 1978). The metabolic quotient (qCO_2) and the C_{mic}/C_{org}-ratio are considered to indicate ecosystem disturbances and are calculated from the basal respiration/microbial biomass ratio (mg CO_2-C g^{-1} C_{mic} h^{-1}) and the ratio of microbial biomass C to organic C (mg C_{mic} $g^{-1}C_{org}$).

To investigate changes of the physiological abilities of the microbial community, Biolog GN microtiter plates containing 95 different C sources and a redox dye (Biolog Inc., Hayward, Ca., USA) (GARLAND & MILLS 1991; modified after INSAM & al. 1995) were used. A representative portion of the soil (10 g) was extracted by shaking for 1h at 220 rpm with 90 ml 0,85 % NaCl. Of the suspension, cell density was determined microscopically after staining with acridine orange (BINNERUP & al. 1993). The plates were inoculated with appropriately diluted suspensions (0.85 % NaCl) to yield an initial cell density of 40000 per well. The plates were then incubated for 11 days at 14 °C. Color formation was measured with a microplate reader (SLT, Grödig, Austria) at 592 nm at least once a day after color formation was observed for the first time. Those readings were selected for further analyses that showed an average absorption value of 1.0 or at least 10 wells exceeded an absorption of 2.0. If neither of these criteria was met, the reading on day 11 was used for further analyses. Before statistical analysis the raw (absorption) data were transformed by calculating the average well color development (AWCD) (GARLAND & MILLS 1991) in a slightly modified way: instead of the C-free well, the well with the lowest absorption value was used as control (INSAM & al. 1996a).

Statistics

For the 1992 samples, 4 replicates were available per plot. In 1993 and 1995, two samplings were made. After an ANOVA had shown that there was no effect of season, the data for the two samplings of the years 1993 and 1995 were pooled, so that for later statistical analyses, 12 replicates were available per year. Differences among treatments were analysed with a one-way ANOVA. Substrate utilization assays were analysed with discriminant analysis (SPSS, Statistical Package of the Social Sciences).

Results

Basal respiration

Basal respiration ranged from 2.5 to 9.0 µg CO_2 g^{-1}soil h^{-1} and from 0.5 to 7.5 µg CO_2 g^{-1} soil h^{-1} for the upper and lower soil horizon, respectively (Fig. 1). In no instance, significant differences between "grazed" and "not grazed" treatments were observed. At the second and third sampling dates, the forest sites had a slightly (not significantly) higher basal respiration than the pasture sites. Basal respiration of the fenced-in forest plot was slightly higher than that of the open forest.

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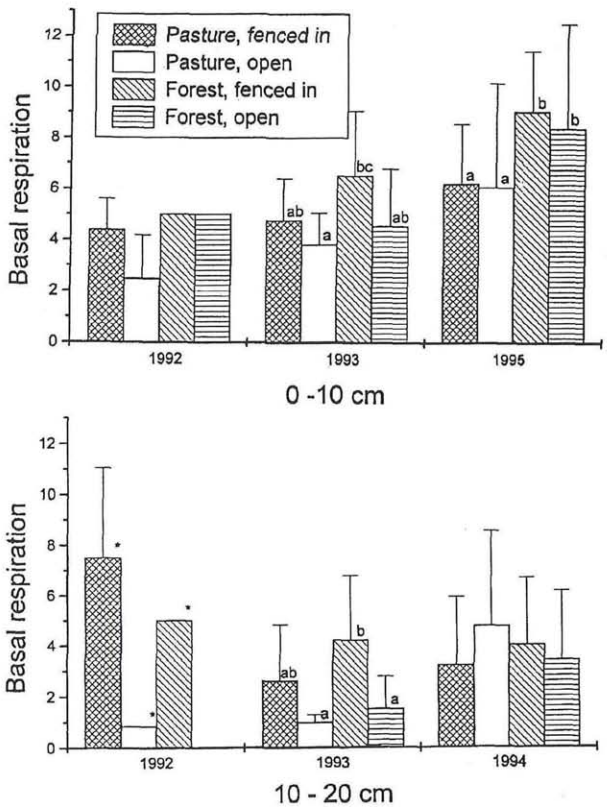

Fig. 1. Basal respiration [µg CO_2 g^{-1} soil h^{-1}] for the soils sampled in 1992, 1993 and 1995 (Mean ± standard deviation). Different letters (a, b, c) indicate significant ($P < 0.05$) differences between treatments within one year.

For the 10 - 20 cm horizon not in all cases were samples available (the soil was too shallow). Thus, for the samples of 1992 no statistical comparison was possible. In 1993, the fenced in plots of both pasture and forest showed an elevated basal respiration. This was, however, not observed in 1995.

Microbial biomass

The microbial biomass showed similar trends as the basal respiration. Microbial biomass was around 1000 and 350 µg C_{mic} g^{-1} in the upper and lower soil horizon, respectively (Fig. 2). At the first sampling date (four replicates only), the open pasture had a much lower biomass than the other treatments. This was not found for the later samplings. As with basal respiration, microbial biomass was slightly higher for the forest plots than for the pasture plots, with a tendency of elevated biomass for the fenced-in sites.

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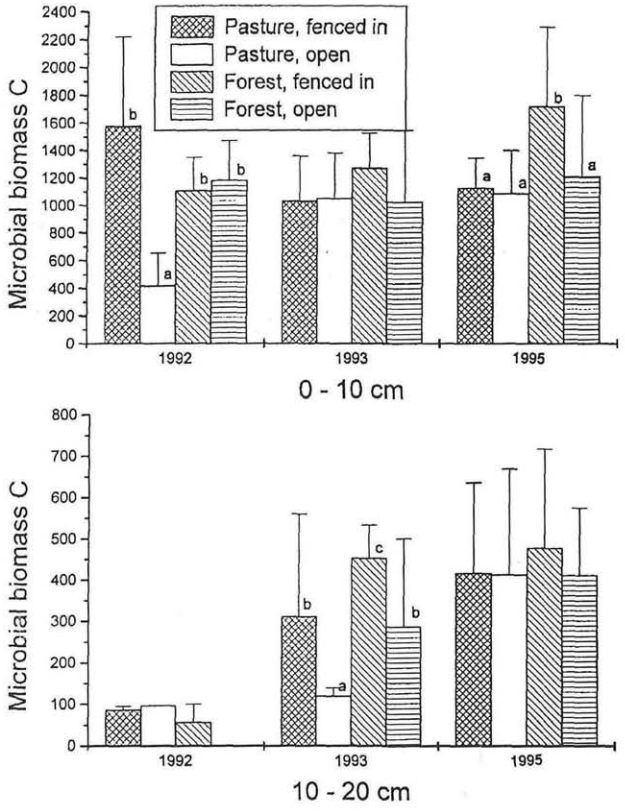

Fig. 2. Biomass [µg C_{mic} g^{-1} soil] for the soils sampled in 1992, 1993 and 1995 (Mean ± standard deviation). Different letters (a, b, c) indicate significant ($P < 0.05$) differences between treatments within one year. Above: upper horizon (0-10 cm), below: lower horizon (10-20 cm).

For the lower horizon, microbial biomass was very low in 1992. In 1993, C_{mic} was higher for both the protected pasture and forest areas when compared to the respective unprotected areas. In 1995, no differences between treatments were found.

Metabolic quotient

Except for the upper horizon in 1992, when the unprotected pasture had a higher qCO_2 than the protected plot, no significant differences were found. For the lower horizon, generally higher values than for the upper horizon were found. However, the variablity was very high and therefore the data are not given here.

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Organic C

Soil organic C contents were between 9 % and 15 % in the upper horizon. Only in 1995, differences between pasture and forest sites were found (Fig. 3). In no case, the differences between fenced in and open areas were significant. In the lower horizon, C_{org} contents ranged from 3 - 8 %, and also there no significant differences were observed.

$C_{mic}:C_{org}$ - ratio

At the first sampling date, the C_{mic}/C_{org} - ratios in the upper horizon at the control pasture (with grazing) was significantly lower than for all other plots (Fig. 4). This, however, can not be explained. All further samples did not show any differences between control and protected areas. Compared with other forest ecosystems, the C_{mic}/C_{org} - ratio in the upper horizon was relatively low (averaging < 10mg C_{mic} g^{-1} C_{org}). This is more characteristic of forest than of pasture sites. The C_{mic}/C_{org} -ratio in the lower horizon were only about half of those in the upper horizons. In 1993, a tendency of an increase in the C_{mic}/C_{org} - ratio in the fenced in subplots of pasture and forest was found. This was, however, not observed in 1995.

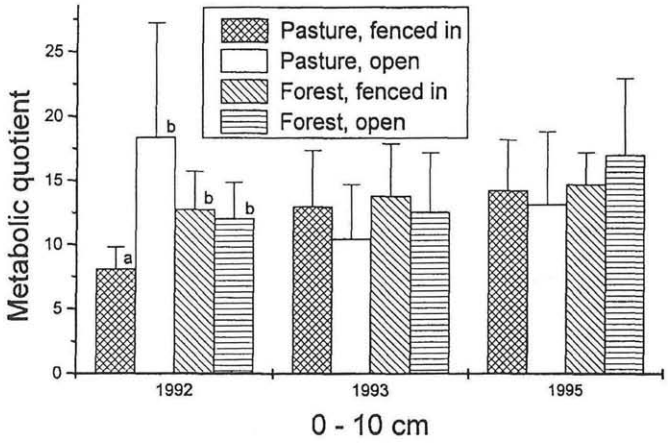

Fig. 3. Metabolic quotient qCO_2 [µg CO_2-C μg^{-1} C_{mic} h^{-1})* 10^{-4}] for the soils sampled in 1992, 1993 and 1995 (Mean ± standard deviation). Different letters (a, b) indicate significant ($P < 0.05$) differences between treatments within one year. Only upper horizon shown (0 -10 cm).

Substrate utilization assay

With the Biolog® substrate utilization assay it was attempted to detect differences in the metabolic abilities of the microbial communities. A canonical discriminant analysis (CDA) was able to separate the samples of the different plots from each other (Fig. 5, 6), for the upper as well as for the lower horizon. In the upper horizon, function 1 separated the open pasture plot very well from the other

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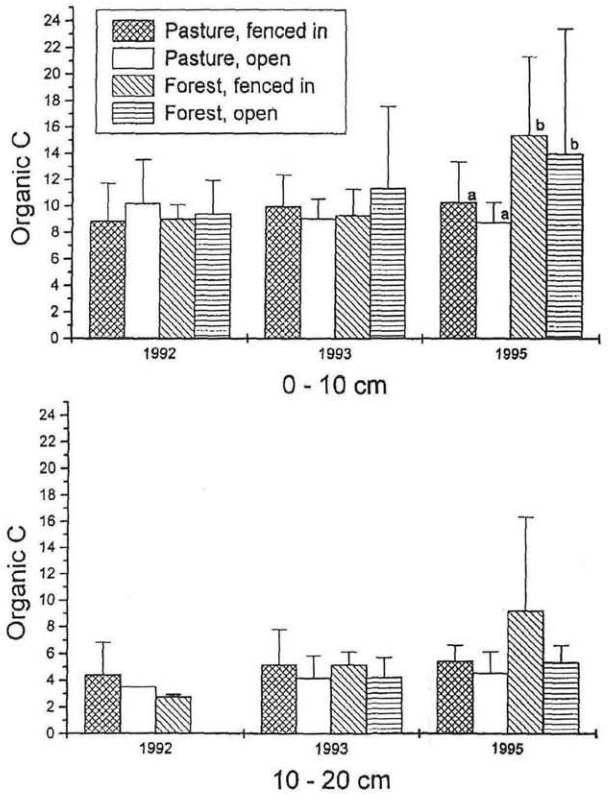

Fig. 4. Organic carbon (% C_{org}) for the soils sampled in 1992, 1993 and 1995 (Mean ± standard deviation). Different letters (a, b) indicate significant ($P < 0.05$) differences between treatments within one year. Above: upper horizon (0-10 cm), below: lower horizon (10-20 cm).

plots. According to the correlation analysis (Table 1), amino acids like proline and leucine were positively correlated with function 1, while sorbitol, methylglycoside, butanediol and inosine were negatively correlated with function 1. The remaining plots were further separated by function 2. As a general trend, the scores of function 2 were lower for the fenced in plots than for the open plots. For the lower horizon, both open plots were well separated from the fenced in plots. The fenced-in pasture samples were most clearly separated from the other samples. Proline, hydroxybutyric and aspartic acid as well as butanediol, ornithine and asparagine showed high correlations with function 1. Inosine and citric acid showed the highest positive correlation with function 2.

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Table 1. Correlations between discriminating functions and substrates (top: upper horizon; bottom: lower horizon).

Upper horizon	**Coefficient of correlation**	
Carbon source	function 1	function 2
D-sorbitol	-0.48	
ß-methyl-glucoside	-0.45	
2-3- butanediol	-0.41	
Inosine	-0.40	
L-proline	0.36	
L-leucine	0.34	
Glucose-1-phosphate		0.61
L-phenylalanine		0.45
Glucuronamide		0.45
L-rhamnose		0.41
Lower horizon	**Coefficient of correlation**	
Carbon source	*function 1*	*function 2*
L-proline	0.57	
γ-Hydroxybutyric acid	0.55	
L-Aspartic acid	0.51	
2-3- Butanediol	0.49	
L-ornithine	0.44	
L-asparagine	0.40	
Inosine		0.38
Citric acid		0.38
L-rhamnose		-0.36
Sucrose		0.33

Discussion

The carbon status of soils is dependent on the input as well as the metabolic activity of the microorganisms. Microbial biomass, and especially its ratio to organic carbon (POWLSON & al. 1987, INSAM & DOMSCH 1988), is an early indicator for changes in the carbon status of soils. KIEFT 1994 found a decrease in biomass and the C_{mic}:C_{org} ratio 11 years after the end of grazing in a comparable ecosystem which he explained by an increased allocation of C to the belowground pool upon grazing. For an abandoned meadow at the Monte Bondone (Italy), INSAM & al.. (unpublished) found a lower biomass and a lower C_{mic}:C_{org} ratio than for a pasture (grazing) and a meadow (grass cutting). The explanation was a decreased degradability of the produced organic substances, a conclusion that was supported by the increased xylanase activity. RUESS & MCNAUGHTON 1987 reported that intensively grazed grassland had higher C_{org} and C_{mic} contents than control grassland. On the other hand, HOLLAND & DETLING 1990 and PANDEY & SINGH 1992 found that grazing reduced belowground biomass. On the sites studied here, SOBOTIK & POPPELBAUM 1994 found slightly elevated fine-root biomass on

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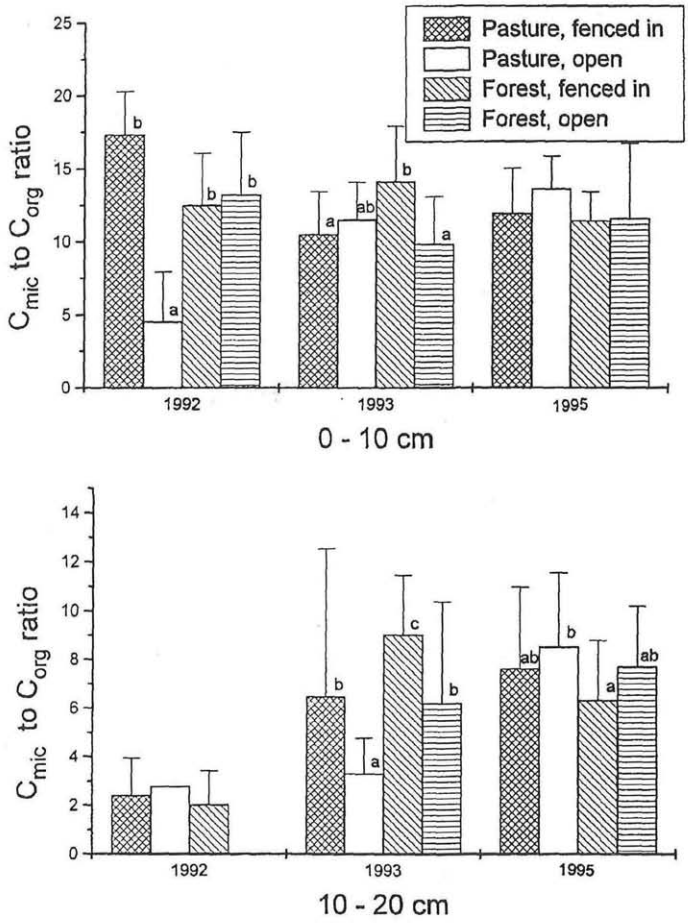

Fig. 5. C_{mic}:C_{org} ratio [mg C_{mic} g^{-1} C_{org}] for the soils sampled in 1992, 1993 and 1995 (Mean ± standard deviation). Different letters (a, b, c) indicate significan ($P < 0.05$) differences between treatments within one year. Above: upper horizon (0-10 cm), below: lower horizon (10-20 cm).

the fenced in forest site, and significantly longer root hairs on the fenced in pasture site as compared to the open pasture site. Both indicates a slight increase of belowground input. We did not find any significant differences in microbial biomass or the C_{mic}:C_{org} ratio between treatments that would have been constant throughout the period of observation. The significant effect on C_{mic} on the forest plot in 1995 is surprising since this plot had only be fenced in in 1992. It is thus possible that the effect was due to the fall of a major spruce tree after heavy

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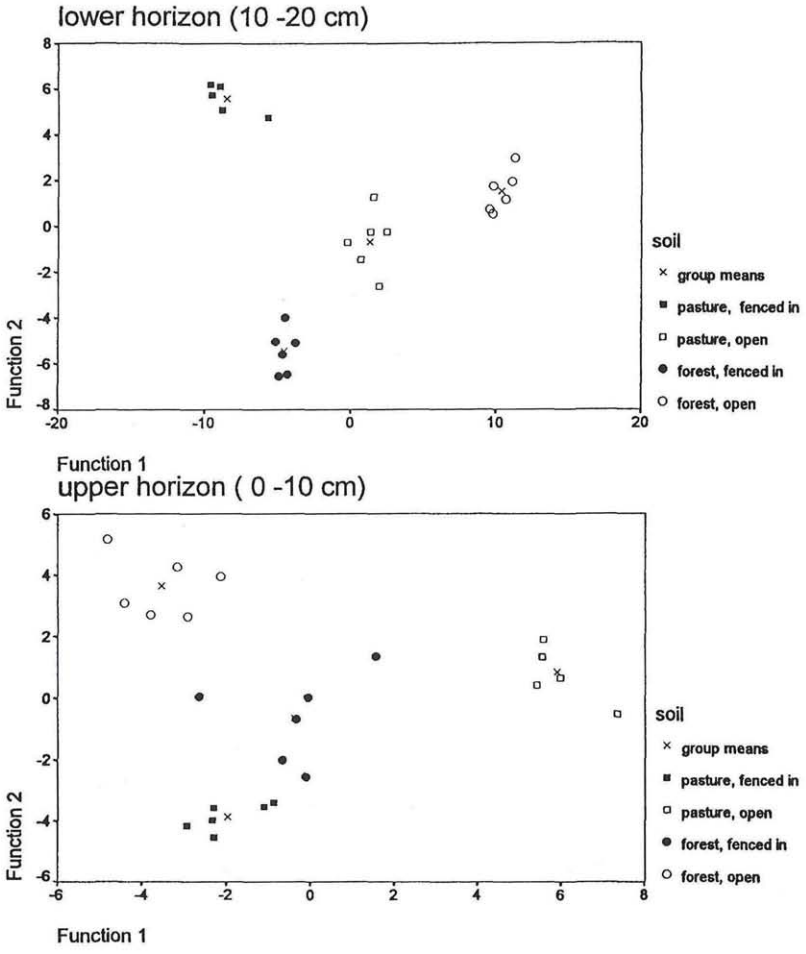

Fig. 6. Ordinate plot after discriminant analysis of the Biolog® substrate utilization assay. The further distant the single clusters are, the more different are the substrate use abilities of the bacterial communities of the 4 treatments. Top: samples from the upper horizon; bottom: samples from the lower horizon.

snowfall in the preceding winter. The tree fell right across the plot and may have caused some microclimatic changes.

The lack of consistant changes in this study may be due to the very heterogenic sites that resulted in a high variability. Considering that consequences of abandoning pastures are long-term, it is not probable that the effects (decrease) observed with C_{mic} and basal respiration in the second year are typical. More likely, these effects were determined by the weather conditions in the two years of

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observation. In 1993 the summer was warm and dry, while the summer in 1995 it was cold and moist. Especially in the months prior to sampling in 1995, the precipitation was extraordinary high. It may be concluded that the effect on the soil (microbial) C status and turnover is especially high when the conditions are dryer, as has earlier been observed.

A comparison with data in the literature shows that our C_{mic}:C_{org} ratios are more typical for a forest than for a grassland site. This may be explained by the fact that the pasture had been forested prior to its use as a pasture. It is thus also difficult to draw conclusions on the humus dynamics of the soils. The qCO_2 did not show any significant differences between grazed and fenced-in sites. KIEFT 1994 found an increase in the qCO_2 11 and 16 years after the end of grazing, as did INSAM & al. (unpublished), who found a higher qCO_2 on an abandoned pasture than on a pasture with grazing. No such consistent effects were found here.

The investigation of substrate utilization patterns offers the possibility of opening up the microbial 'black box'. Even if changes on the bulk biomass and turnover parameters and the ecophysiological quotients do not occur, it is possible that functional abilities change. The results as shown in Fig. 3 clearly indicate that some functional shifts occurred that may be attributed to a cessation of grazing. On the open pasture, proline and leucine seem to be more common microbial substrates than on the other plots, while N-free C sources like sorbitol, methylglucoside, butanediol and inosine probably are less abundant on these plots. These changes may be attributed to a shift in the quality of organic matter input, i.e. a change in the composition of substrates available to the bacteria. Since no data are available on the chemical composition of root exudates or plant litter, the observed differentiation of the microbial communities by utilization of certain C sources cannot be attributed to a specific change in the plant community. It may for example be the consequence of the shift from grasses to shrubs as observed by SOBOTIK & POPPELBAUM 1994, or to a change in the shoot/root ratio. With a similar approach, ZAK & al. 1994 found distinct shifts in bacterial substrate utilization patterns and microbial diversity under different kinds of desert shrub plants. For investigating effects of land-use change and pasture abandonment, the Biolog® approach has for the first time been used in the present study.

Summarized, the results suggest that the effects of abandoning grazing of alpine grassland on microbial biomass and basal respiration are small, and are more pronounced in dry than in moist years. Further, there seems to be an effect on microbial substrate utilization patterns, which probably is due to a change of organic matter input quality.

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