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# Effects of mineral and organic fertilizers on the microfauna in a high-altitude reafforestation trial

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**Summary.** Protozoa (testate amoebae, ciliates), small metazoa (rotifers, nematodes), and soil enzymes (catalase, cellulase) were investigated in a reafforested fertilized site at the alpine timberline. Side-dressings of mineral and organic fertilizers were applied alone (90 g NPK; 90, 180, 300, and 450 g dried bacterial biomass per spruce seedling) and in combination with magnesite (90 g NPK + 300 g Mg; 90, 180, and 300 g bacterial biomass + 300 g Mg each; 30 g dried fungal biomass + 270 g Mg). One-third of each quantity was applied in 1986, and two-thirds in 1988. None of the treatments caused a significant decrease in the biological parameters investigated in comparison with untreated controls. The soil life was more or less stimulated depending on the amount of organic material contained in the fertilizers and the quantity; 180–270 g organic material per seedling proved to be the most effective. Dried bacterial biomass increased the pH by about 0.5 units, catalase activity by about 70%, and the number of ciliates and nematodes by 150–400%. The ciliate biomass and the number of ciliate species were likewise increased. The organomineral fertilizers increased pH by up to two units and also stimulated the soil life, but where the organic content was less than 180 g per seedling, efficiency decreased markedly. The least biological activity was observed in the control soil and in soil fertilized with NPK. Testaceans, rotifers, and cellulolytic activities were only slightly (insignificantly) affected by the treatments. A pooled evaluation of the data (organic versus organomineral versus mineral treatments) and community analyses showed that the organic fertilizer caused a more pronounced increase in the soil life and greater changes in community structure than the mineral combinations. Two years after application of the fertilizers, the differences between the treatments and the unfertilized controls had diminished.

**Key words:** Protozoa – Nematoda – Rotatoria – Soil enzymes – Organic fertilizers – Subalpine soil – Reafforestation

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The major problem of reafforestation at the timberline is the transplantation shock suffered by seedlings under the extreme climatic conditions. A proper nutrient supply is needed to avoid a decline in the annual biomass increment and thus incomplete maturation, which enhances the risk of frost desiccation (Tranquillini 1979; Glatzel and Fuchs 1986). Pot experiments have shown that certain organic fertilizers allow better growth of spruce seedlings than conventional NPK fertilizers (Glatzel and Fuchs 1986). This has been confirmed by studies on revegetated ski slopes (Köck and Holaus 1981; Insam and Haselwandter 1985; Lüftenegger et al. 1986). The present experiment was designed to study the effects of various fertilizers on the growth of spruce seedlings under field conditions (Glatzel et al. 1991). This paper is concerned with the quantitative and qualitative changes of the micro-edaphon. A further paper will deal with the autecology of certain protozoan species.

## Materials and methods

### *Site description and experimental design*

The study was performed on the Gressensteinalm (Wildschönau, Tyrol; 12° 05′ E, 47° 40′ N), 1800 m above sea level. The experimental area is on a southeast-facing slope (inclination 50–60°) in the dwarf pine zone. About 40 years before the present investigation the dwarf pines (*Pinus mugo mugo*) were felled for oil production. Old tree stumps and clumps of Norway spruce (*Picea abies*) indicate that the natural timberline is at about 2100 m at this site. The soil is an iron humus podsol on quartz phyllite.

About 430 three-year-old pot-grown ("paper pot") Norway spruce seedlings were planted in holes (13 cm deep and at least 1 m apart) together with the paper pot. Treatments were assigned at random to each seedling. The fertilizers and quantities applied are summarized in Tables 1 and 2. Bactosol (Biochemie GmbH, Kundl, Austria) is a fermentation byproduct made from dried bacterial biomass. Biomag (Tiroler Magnesit AG, Hochfilzen, Austria) consists of 80% crude magnesite ($MgCO_3$), 10% caustic magnesite [$CaMg(CO_3)_2 + MgO$], and 10% biosol (dried fungal biomass produced by Biochemie GmbH, Kundl, Austria). The fertilizers were spread in granular form around the seedling (∅ ca. 20 cm). The seven bactosol treatments were applied immediately after planting in June 1986. NPK and magnesite, as mineral fertilizers,

were side-dressed 6 weeks later. The biomag treatment was applied in 1987. All treatments were reapplied with double quantities in June 1988. The experiment was planned and conducted by the Institut für Forstökologie, Universität für Bodenkultur, Vienna (Glatzel et al. 1991).

### *Sampling and counting procedures*

Samples were taken on 6 September 1986, 19 September 1987, 28 September 1988, 16 September 1989, 25 June 1990, and 4 October 1990. On each sampling day one seedling from each of the 11 treatments was selected at random, so that a total of 65 different seedlings (treatment G missing in 1986) was investigated. About eight soil cores were taken with a steel corer (∅ 3 cm) from a soil depth of 0–10 cm around each seedling. These eight cores were thoroughly mixed in the laboratory and pooled into one sample. Direct counting according to Lüftenegger et al. (1988) was used to enumerate testaceans, nematodes, and rotifers, while a culture method (Foissner 1987a) was used to estimate the potential abundance of the ciliates. The biomass was calculated as described in Foissner (1985).

### *Abiotic factors and soil enzymes*

Soil moisture was evaluated after air-drying (about 4 weeks at room temperature). In the air-dried samples, pH (glass electrode; 0.01 *M* $CaCl_2$) and catalase and cellulolytic ("cellulase") activity were determined according to Beck (1971) and Hofmann (1979), respectively.

### *Statistics*

Although the arithmetic means obtained from the six sampling dates were quite different, few were significantly different ($P<0.05$) due to a high level of variation between blocks (years), arising from very low mean values after planting and considerable annual fluctuations [see standard deviation of unfertilized control (A); Table 3]. In addition, the large number of treatments increased the variance. Thus it was necessary to construct more uniform groups (Steel and Torrie 1980; Mulla et al. 1990). We examined the relative values of biological variables, dividing each value by the mean of all samples ($n = 11$) taken on the same day. This kind of homogenization minimizes the variance and reduces the probability of a type 2 (beta) error, which means that acceptance of the null hypothesis is invalid (Schuster and Lochow 1979; Moder 1990). Although this practice may be rejected from a statistical point of view due to the biased variance estimators (Moder 1990), compared with the absolute values, the relative (biased) data did not cause unreliable treatment effects. All data shown in the tables are unbiased to facilitate the comparison. The missing value in 1986 for treatment G was interpolated from the mean of the five available values in order to simplify the analysis of variance; it was omitted in the pairwise and group comparisons.

The data were examined with a two-way analysis of variance. Where necessary, values were square-root transformed to meet the assumptions of this procedure (normality was examined with the Kolgomorov-Smirnov test and homogeneity of variances with the Cochran test; Sachs 1984). If a treatment effect was suggested, pairwise comparisons between the control and each treatment using least significant differences (LSD) followed. Groups of fertilizers were compared by partitioning the experimental error of the analysis of variance (e.g., Steel and Torrie 1980).

The Soil Biological Index, a quantitative general estimate of treatment effects, was calculated according to Wodarz et al. (1992) from the total abundance, the dominance structure, the species number, and ecological weights (habitat preference and position of species in the *r/K* continuum; testacean species were also classified according to pH preference). With testaceans, low index values indicate natural (e.g., undisturbed) or improved soil conditions. With ciliates the index demands a different interpretation, because natural, evolved soils contain very few active ciliates due to the suppressive effects of ciliatostasis (Foissner 1987b; Petz and Foissner 1988); thus a low index indicates unnatural (e.g., disturbed) or extreme soil conditions (e.g., litter layer).

The indices of Jaccard (1902), Bray and Curtis (1957), and Morisita (1959) were used to measure species and species-abundance similarity. Clusters were constructed by the unweighted pair-group method with arithmetic means.

**Table 1.** Composition of fertilizers (as used; bactosol and biomag have been improved since)

| Fertilizer component | NPK (%) | Bactosol (%) | Biomag (%) | Crude magnesite (%) |
|---|---|---|---|---|
| Organic material | – | 60 | 7[a] | – |
| N | 12 | 4–6[b] | 0.5–0.7[a,b] | – |
| P | 10 | 3–5 | 0.1–0.2[a] | – |
| K | 18 | 3–5 | 0.3–0.4[a] | – |
| Mg | – | 1–3 | 0.05–0.3[a] | – |
| Ca | – | 6–9 | 0.3–0.5[a] | – |
| Magnesite | – | – | 90[c] | 100 |

[a] Equivalents from 10% biosol fertilizer
[b] Organically bound; includes <0.3% soluble N
[c] 80% crude magnesite and 10% caustic magnesite

**Table 2.** Treatments, quantities and year of application of fertilizers

| Treatment | Quantity of fertilizer (g per seedling) and year | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NPK | | Bactosol | | Biomag | | Crude magnesite | |
| | 1986 | 1988 | 1986 | 1988 | 1987 | 1988 | 1986 | 1988 |
| A | – | – | – | – | – | – | – | – |
| B | 30 | 60 | – | – | – | – | – | – |
| C | – | – | 30 | 60 | – | – | – | – |
| D | – | – | 60 | 120 | – | – | – | – |
| E | – | – | 100 | 200 | – | – | – | – |
| F | – | – | 150 | 300 | – | – | – | – |
| G | – | – | – | – | 100 | 200 | – | – |
| H | 30 | 60 | – | – | – | – | 100 | 200 |
| I | – | – | 30 | 60 | – | – | 100 | 200 |
| K | – | – | 60 | 120 | – | – | 100 | 200 |
| L | – | – | 100 | 200 | – | – | 100 | 200 |

## Results

### *pH*

The organomineral fertilizers brought about a highly significant increase in pH at a soil depth of 0–10 cm. Treatments GHIKL (Table 2) differed from the control by about two units, corresponding to a shift from very strongly acid to moderately acid (Tables 3–5). This difference was still evident in October 1990, indicating a long-term effect (Fig. 1a). The maximum difference of 2.9 units was measured after high-rate fertilization in 1988 at the bactosol and magnesite sites (IKL; Fig. 1a). However, the uppermost limit of pH 5.8 recommended for Fe humus podsols to avoid Mn deficiency was not exceeded at any site (Scheffer and Schachtschabl 1982). Three months after fertilization pH ranged from 4.1 to 5.7 in the organic treatments (CDEF; Fig. 1a). These high values cannot easily be explained by the 6% Ca contained in bactosol (Table 1). A more likely explanation is that these paper pot seedlings were cultivated in more neutral (limed) soil, some of which remained attached during planting; this is supported by the weak fertilizer effects seen in Fig. 1a. Omitting the 1986 values, pH was on average 0.5 units higher in the pure organic treatments than in the control (A; Tables 3, 5). Small amounts of NPK (B) had almost no effect on pH (Tables 3, 4).

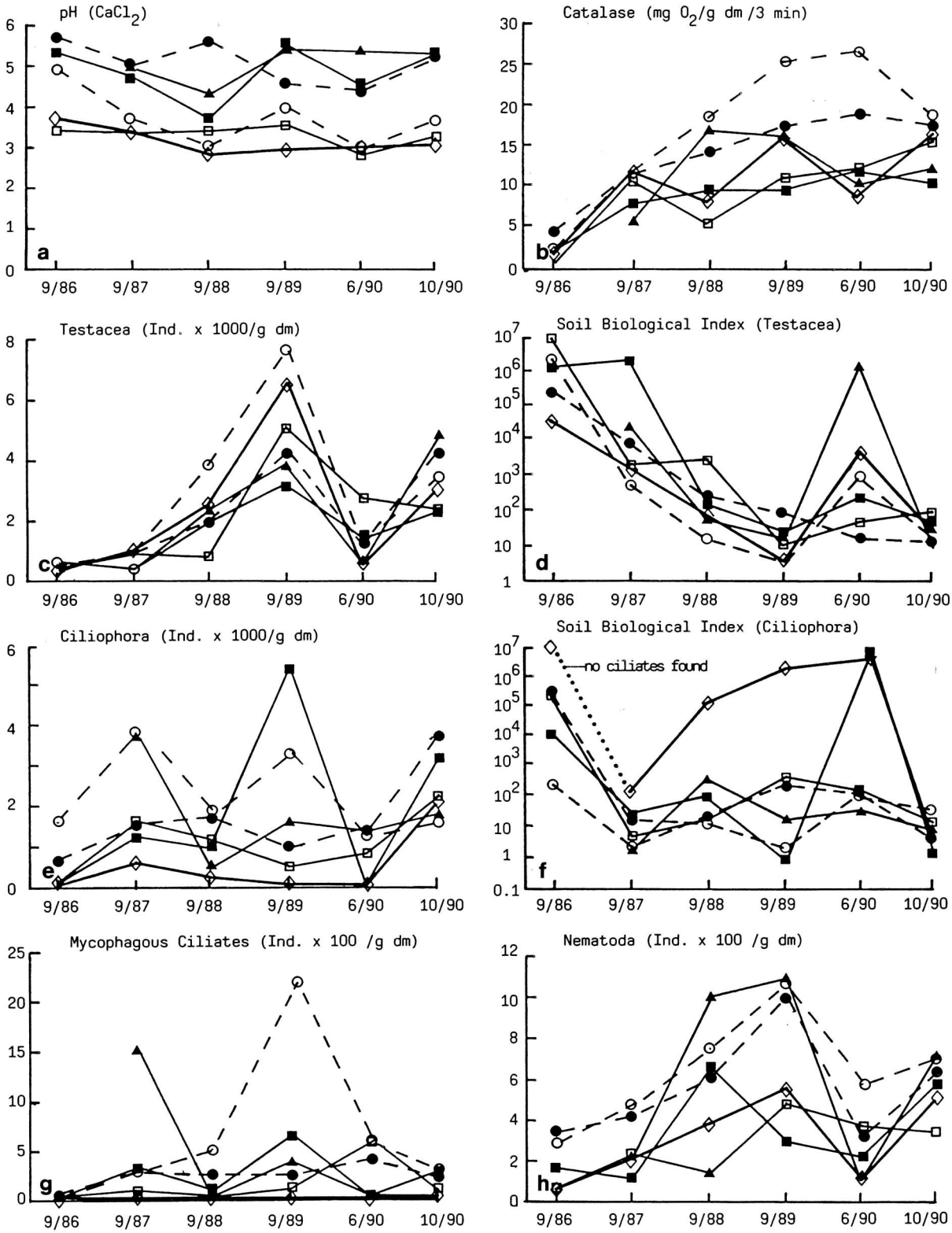


**Fig. 1a–h.** Effects of organic and mineral fertilizers on pH (**a**), catalase activity (**b**), testacean abundance (**c**), testacean Soil Biological Index (**d** logarithmic scale), ciliate abundance (**e**), ciliate Soil Biological Index (**f** logarithmic scale), mycophagous ciliate abundance (**g**), and nematode abundance (**h**). ◇– – – ◇, unfertilized controls (A); ○ – – – ○, bactosol alone (C–F); ● – – – ●, bactosol and magnesite (IKL); ▲ – – – ▲, biomag (G; excluding 1986); □ – – – □, NPK alone (B); ■ – – – ■, NPK and magnesite (H); dm, dry mass of soil; Ind., individuals; month and year of sampling are given. For details of treatments, see Table 2

*Soil enzymes*

Catalase activity increased by 52–84% after application of the organic fertilizer (CDEF; Tables 3, 4). An increased activity, though less pronounced, was also observed in those treatments with higher quantities of bactosol and magnesite (KL; Tables 3–5). However, by October 1990 these treatments were approaching the control level (A), indicating that the additional nutrients had been exhausted (Fig. 1 b). Biomag (G) and the low level of bactosol and magnesite (I) caused insignificant changes (Tables 3, 4). The NPK treatments (BH) even slightly (insignificantly) decreased catalase activity compared to the control (A; Fig. 1 b; Tables 3–5).

Cellulolytic activity showed no significant fertilizer effects, possibly due to the small sample size (not measured in 1986). However, the activity was usually slightly higher in the fertilized treatments than in the control (A; Table 3).

No correlations between enzyme activities and protozoan or nematode abundance were obtained due to inconsistent variations, e.g. different maxima dates (Fig. 1 b, c, e, h). However, Foissner (1985) observed a negative correlation ($P<0.05$) between the species number, abundance, and biomass of testate amoebae and catalase activity in podsolic alpine soils from the Gastein area (Central Alps).

*Testacea*

Abundance, biomass, and species numbers tended to be higher in the organic treatments (DEF) than in the control (A), but were usually lower in the organomineral treatments (BHIK; Fig. 1 c). The differences were statistically insignificant (Tables 3, 4), in agreement with results by Berger et al. (1986) and Lüftenegger et al. (1986). The total abundance values, however, obscured the fact that some species were considerably affected, e.g., the acidophilic *Corythion dubium*, whose numbers decreased significantly in the magnesite treatments (Tables 4, 5). The analysis of community structure similarly suggested some noticeable fertilizer effects, i.e., species-abundance similarity clustered around some magnesite and the pure NPK treatments (Fig. 2 c). Since these treatments shared a lower abundance than the control, NPK and neutralizing fertilizers are obviously somewhat harmful to the testate amoebae. This is substantiated by the increased Soil Biological Index values, which show a distinct disturbance of the testacean community in these soils where pH had increased, especially in treatments BGH (Fig. 1 d; note the logarithmic scale), possibly due to direct toxic effects. However, Chardez et al. (1972) observed a doubling of the testacean abundance 3 years after the application of NPK to a deciduous forest soil. The increase in the index in the present work was mainly caused by the dominance (42%) of *Trinema lineare*, a characteristic colonizer of disturbed biotopes (Lüftenegger et al. 1985, 1986).

Treatment E was distinctly separate in terms of both species-abundance measures and the high individual numbers (Fig. 2 c, e; Tables 3, 4). The species composition, however, was very similar to that of the untreated controls (A; Fig. 2 a). Thus the application of a medium quantity of bacterial biomass seems more beneficial to testaceans than the other treatments.

**Table 3.** Effects of organic and mineral fertilizers on abiotic and biotic parameters

| Parameter | Treatment | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | K | L |
| Soil moisture | 49.2 | 55.3 | 49.3 | 57.1 | 50.9 | 56.8 | 52.8 | 53.3 | 53.2 | 52.9 | 54.1 |
| (% of dm) | 9.9 | 6.3 | 12.0 | 11.8 | 12.7 | 13.0 | 14.2 | 9.0 | 7.8 | 10.7 | 15.0 |
| pH ($CaCl_2$) | 3.0 | 3.3 | 3.2 | 3.2 | 3.8 | 3.6 | 5.0 | 4.8 | 5.0 | 4.8 | 5.1 |
| | 0.2 | 0.3 | 0.6 | 0.5 | 0.6 | 0.4 | 0.5 | 0.7 | 0.6 | 0.6 | 0.8 |
| Catalase activity | 10.8 | 9.5 | 16.4 | 16.3 | 18.0 | 19.8 | 12.6 | 8.8 | 12.8 | 14.9 | 15.7 |
| (mg $O_2$ $g^{-1}$ dm 3 $min^{-1}$) | 5.7 | 5.6 | 10.6 | 10.2 | 10.7 | 11.2 | 4.7 | 3.6 | 4.8 | 6.4 | 7.5 |
| "Cellulase" activity | 8.6 | 8.8 | 10.1 | 14.7 | 12.1 | 13.4 | 10.5 | 9.8 | 11.1 | 9.8 | 12.4 |
| (mg glucose $g^{-1}$ dm 24 $h^{-1}$) | 9.4 | 8.4 | 8.5 | 18.6 | 14.2 | 15.4 | 11.5 | 10.7 | 8.9 | 9.4 | 11.4 |
| Total Testacea | 2282 | 1972 | 2424 | 2747 | 3447 | 2842 | 2301 | 1582 | 1887 | 1632 | 2784 |
| (individuals $g^{-1}$ dm) | 2330 | 1741 | 2164 | 2164 | 4908 | 2499 | 1941 | 1039 | 1640 | 1152 | 2472 |
| Total Testacea | 65.4 | 64.5 | 61.7 | 82.0 | 85.1 | 91.3 | 59.3 | 59.2 | 87.6 | 41.3 | 85.4 |
| (µg biomass $g^{-1}$ dm) | 46.0 | 63.1 | 58.7 | 40.3 | 112.1 | 53.1 | 51.6 | 32.8 | 86.3 | 22.8 | 99.6 |
| Mean Testacea | 14 | 15 | 16 | 20 | 18 | 18 | 17 | 17 | 17 | 16 | 16 |
| (species number) | 6 | 8 | 9 | 5 | 7 | 7 | 7 | 5 | 8 | 5 | 8 |
| Total Ciliophora | 519 | 1067 | 1265 | 2431 | 2758 | 2568 | 1776 | 1837 | 2558 | 1170 | 1308 |
| (individuals $g^{-1}$ dm) | 828 | 759 | 995 | 2373 | 1595 | 1385 | 1154 | 2080 | 2087 | 981 | 788 |
| Total Ciliophora | 6.8 | 19.1 | 32.8 | 44.7 | 64.7 | 37.5 | 46.1 | 50.1 | 38.5 | 23.8 | 25.9 |
| (µg biomass $g^{-1}$ dm) | 8.2 | 17.6 | 37.0 | 51.1 | 41.8 | 28.3 | 24.2 | 44.4 | 19.8 | 20.0 | 17.1 |
| Mean Ciliophora | 10 | 15 | 14 | 15 | 20 | 17 | 15 | 19 | 19 | 13 | 16 |
| (species number) | 8 | 7 | 5 | 6 | 8 | 6 | 9 | 12 | 4 | 6 | 10 |
| Total Nematoda | 309 | 273 | 695 | 539 | 528 | 800 | 502 | 338 | 323 | 589 | 749 |
| (individuals $g^{-1}$ dm) | 204 | 152 | 426 | 433 | 236 | 417 | 486 | 226 | 194 | 266 | 526 |
| Total Rotatoria | 10 | 6 | 19 | 22 | 30 | 41 | 50 | 9 | 19 | 27 | 33 |
| (individuals $g^{-1}$ dm) | 8 | 4 | 19 | 23 | 32 | 64 | 82 | 10 | 38 | 44 | 40 |

Arithmetic means (first line) and standard deviations (second line) are shown. For explanation of treatments, see Table 2. Values for 1986 for treatment G not included, nor 1986 pH nor cellulase activity; dm, dry mass of soil

**Table 4.** Abiotic and biotic parameters of fertilized treatments in relation to control (A in Table 3 = 100%)

| Parameter | Treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | B | C | D | E | F | G | H | I | K | L |
| pH ($CaCl_2$) | 8 | 6 | 8 | 26* | 20$^{+}$ | 67*** | 57*** | 64*** | 59*** | 68*** |
| Catalase activity ($mg\ O_2\ g^{-1}\ dm\ 3\ min^{-1}$) | −12 | 52$^{+}$ | 52$^{+}$ | 67* | 84** | 17 | −18 | 19 | 38$^{+}$ | 46* |
| Total Testacea (individuals $g^{-1}$ dm) | −14 | 6 | 20 | 51 | 25 | 1 | −31 | −17 | −28 | 22 |
| Testacea (*Corythion dubium*) (individuals $g^{-1}$ dm) | 25 | 93 | 1 | −11 | 1 | −70$^{+}$ | −58$^{+}$ | −74$^{+}$ | −67$^{+}$ | −40$^{+}$ |
| Total Ciliophora (individuals $g^{-1}$ dm) | 105 | 144* | 368** | 431*** | 395*** | 242* | 254* | 393*** | 125 | 152* |
| Total Ciliophora (µg biomass $g^{-1}$ dm) | 179 | 379* | 553* | 845*** | 447* | 573** | 632** | 462** | 247 | 278* |
| Mean Ciliophora (species number) | 48* | 35* | 45* | 89*** | 60** | 47** | 82** | 87*** | 26$^{+}$ | 60* |
| Ciliophora (Colpodea) (individuals $g^{-1}$ dm) | 164 | 450 | 1226* | 1155* | 1115$^{+}$ | 609 | 412 | 979$^{+}$ | 171 | 416 |
| Ciliophora (Grossglockneridae) (individuals $g^{-1}$ dm) | 991 | 681 | 5203* | 2983 | 8159*** | 2491 | 1474 | 1838 | 848 | 2226 |
| Total Nematoda (individuals $g^{-1}$ dm) | −12 | 125** | 74[a] | 71$^{+}$,[a] | 159*** | 62 | 9 | 5 | 90* | 142** |

Data represent percentage difference. For explanation of treatments, see Table 2. $^{+}P<0.1$, $*P<0.05$, $**P<0.01$, $***P<0.001$, by least significant difference

[a] Comparison inconsistent due to homogenization; dm, dry mass of soil

**Table 5.** Comparison of abiotic and biotic parameters in pooled treatments

| Parameter | Pooled treatments vs. control | | | | | | | | Bac vs. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | Fert | NPK | Bac | Bac+Mg | Mg | <dose | >dose | Om | Bac+Mg |
| Sample size | 6 | 59 | 12 | 24 | 18 | 29 | 29 | 30 | 24/35 | 24/18 |
| pH ($CaCl_2$) | 3.0 | 4.2*** | 4.0** | 3.5* | 4.9*** | 4.9*** | 3.7** | 4.6*** | 4.6*** | *** |
| | 0.4 | 0.9 | 0.9 | 0.6 | 0.6 | 0.6 | 0.8 | 0.8 | 0.8 | |
| Catalase | 10.8 | 14.5$^{+}$ | 9.2 | 17.7* | 14.5* | 13.0 | 14.7 | 14.4$^{+}$ | 11.8** | |
| ($mg\ O_2\ g^{-1}\ dm\ 3\ min^{-1}$) | 5.7 | 8.2 | 4.5 | 10.0 | 6.1 | 5.8 | 8.8 | 7.3 | 5.8 | |
| Testacea (*Corythion dubium*) | 227 | 182 | 193 | 276 | 75$^{+}$ | 82$^{+}$ | 253 | 112 | 118** | *** |
| (individuals $g^{-1}$ dm) | 214 | 250 | 266 | 312 | 87 | 105 | 309 | 124 | 174 | |
| Total Ciliophora | 519 | 1875*** | 1452$^{+}$ | 2256*** | 1679** | 1728** | 1862** | 1888** | 1602* | |
| (individuals $g^{-1}$ dm) | 828 | 1544 | 1546 | 1663 | 1470 | 1509 | 1544 | 1623 | 1423 | |
| Total Ciliophora | 6.8 | 38.2*** | 34.6* | 44.9** | 29.4** | 36.5** | 41.3** | 35.1** | 31.8 | |
| (µg biomass $g^{-1}$ dm) | 8.2 | 32.7 | 36.1 | 39.7 | 19.1 | 27.3 | 37.5 | 32.7 | 26.5 | |
| Mean Ciliophora | 10 | 17*** | 17** | 16*** | 16** | 17*** | 16*** | 17*** | 16 | |
| (species number) | 8 | 7 | 10 | 6 | 7 | 8 | 6 | 8 | 8 | |
| Ciliophora (Colpodea) | 153 | 1161** | 593 | 1621*** | 950* | 938* | 1228** | 1098* | 861** | * |
| (individuals $g^{-1}$ dm) | 170 | 1310 | 665 | 1422 | 1427 | 1234 | 1261 | 1360 | 1144 | |
| Ciliophora (Grossglockneridae) | 15 | 410* | 195 | 639** | 255* | 271* | 377* | 441** | 274 | |
| (individuals $g^{-1}$ dm) | 8 | 699 | 229 | 1000 | 236 | 323 | 690 | 385 | 308 | |
| Nematoda | 309 | 543* | 306 | 641** | 554* | 517$^{+}$ | 525$^{+}$ | 560* | 429** | |
| (individuals $g^{-1}$ dm) | 204 | 371 | 186 | 379 | 382 | 372 | 364 | 333 | 355 | |

Data show arithmetic means (first line) and standard deviations (second line) of six sampling occasions and are compared by partitioning the experimental error of the two-way analysis of variance

A, untreated control; Fert, fertilized treatments (B – L); NPK, mineral treatments (BH); Bac, bactosol treatments (CDEF); Bac + Mg, bactosol and magnesite treatments (IKL); Mg, magnesite-dominated treatments (GHIKL); <dose, 90 – 300 g fertilizer per seedling (BCDEG); >dose, 450 – 600 g fertilizer per seedling (FHIKL); Om, organomineral treatments (BGHIKL); for details of treatments, see Table 2; for other explanations, see Table 4

*Ciliophora*

The abundance, biomass, and species numbers of ciliated protozoa increased significantly in most treatments. Compared to the control (A), pure bactosol (EF) caused the greatest and NPK (BH) the smallest changes (Fig. 1 e, 2 d, f; Tables 3 – 5). The high abundance in fertilized treatments was mainly due to the colpodids (Tables 4, 5), a group of specialized, *r*-selected ciliates which colonize disturbed and/or extreme biotopes (Lüftenegger et al. 1985). Four colpodid (*Colpoda inflata*, *C. maupasi*, *C. steinii*, *C. cucullus*), a hypotrich (*Gonostomum affine*), and a microthoracid species (*Leptopharynx costatus*) contributed most to the increased abundance. These species are strictly bacterivorous, indicating an increased supply of bacteria. The mycophagous colpodids of the family Grossglockneridae were significantly increased in the organically fertilized treatments (DF; Fig. 1 g; Tables 4, 5), indicating an increased fungal abundance. This group showed maximum values 1 year after the fertilizer applications, and then its dominance decreased slightly until the end of the investigation period. However, their numbers were still five times higher than in the control (A; Fig. 1 g).

The changes in ciliate abundance and species composition deviated clearly from control levels (A) in the similarity measures (Fig. 2 b, d, f). The species composition in the pure NPK treatments (B) was very similar to that of the controls (A; Fig. 2 b), again suggesting that the NPK fertilization was ineffective. The organic and organomineral treatments were fairly consistently grouped into two further clusters (Fig. 2 b). In the species-abundance similarity, the comparatively weak effects of low quantities of bactosol and magnesite (IK) and of both NPK treatments (BH) were clearly recognizable (Fig. 2 d, f). Moreover, the intermediate position of the biomag and high bactosol + magnesite treatments (LG) was remarkable (see ranking of fertilizers). The pronounced changes in the ciliate community caused by fertilization were also evident from the markedly decreased values of the Soil Biological Index (Fig. 1 f; see Methods for interpretation). The outlier in treatment H in June 1990 was due to a very low total abundance and a low species number (Fig. 1 e).

The significant increase in ciliate abundance, biomass, and species number, particularly after the application of organic fertilizer, agrees well with results reported by Lüftenegger et al. (1986), who studied revegetated ski slopes. Foissner (1981) likewise found higher abundances

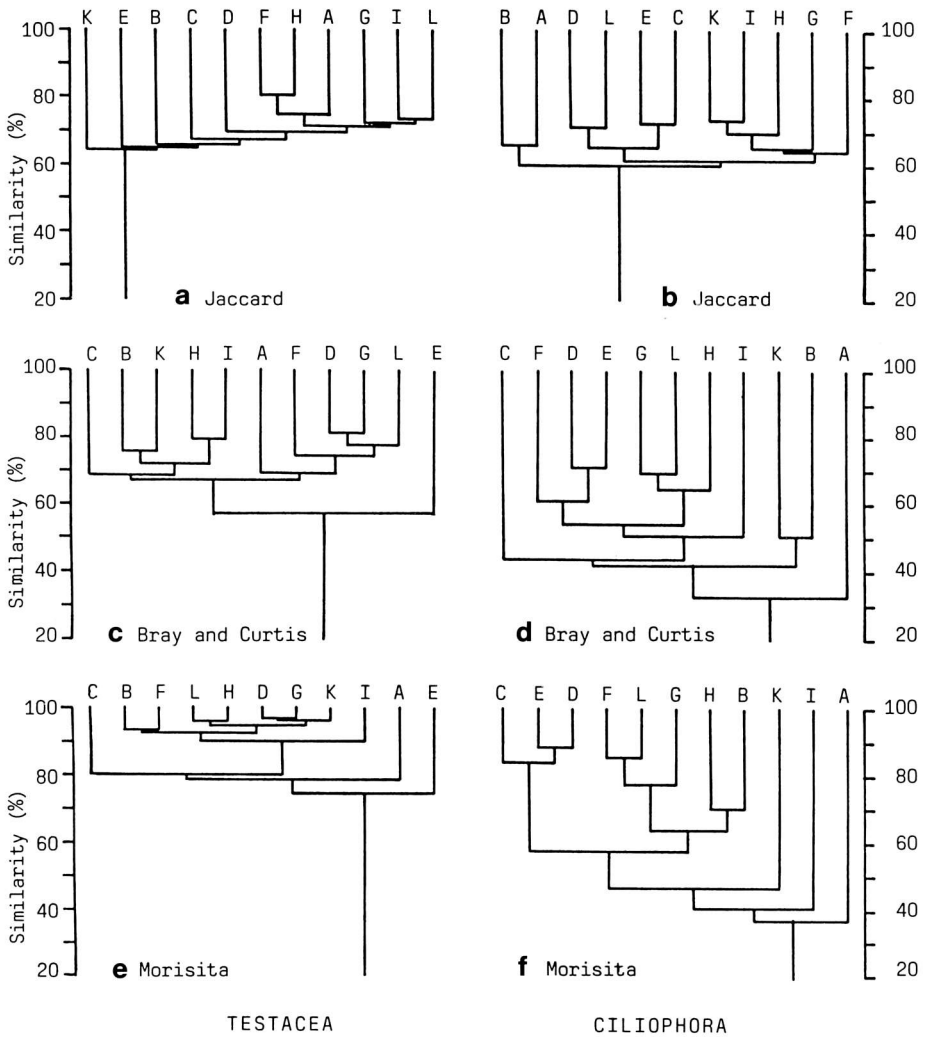


**Fig. 2 a – f.** Similarity in species composition (**a, b** method, Jaccard 1902) and species-abundance similarity (**c, d** method, Bray and Curtis 1957; **e, f** method, Morisita 1959) of protozoans in 11 treatments. Treatments: A, unfertilized controls; CDEF, organic treatments; BGHIKL, organomineral treatments (for details see Table 2)

and species numbers in manured and fertilized alpine pastures than in natural sites. In another alpine pasture, liming also increased the biomass significantly; NPK and ammonium sulphate, however, caused only small changes, while thomasphosphate (14.5% $P_2O_5$, 45% CaO) decreased ciliate abundance (Berger et al. 1986). Fertilizer effects on protozoa in other habitats, e.g., arable land, have been reviewed in detail by Foissner (1987b).

*Nematoda and rotatoria*

The nematode abundance was significantly increased by bactosol with or without magnesite (CEFKL; Tables 3, 4). The pooled comparison showed that higher quantities of fertilizers were slightly more effective than lower levels (Table 5). Compared to the control (A), pure bactosol (CDEF) caused a more pronounced increase ($P<0.01$) than bactosol with magnesite (IKL; $P<0.05$), although there was no statistically significant difference between the two types of bactosol treatment (Table 5). Insignificant effects were found in the NPK and biomag treatments (Tables 4, 5), and nematode numbers were only slightly above the control (A) level at the end of the investigation period (Fig. 1h).

The rotifer abundance was lowest in the NPK treatments (BH), followed by the control (A; Table 3). On the last three sampling dates, numbers in all other fertilized treatments were two to five times higher than in the control. The differences were not significant, however, due to the high variation.

Work on revegetated ski slopes has shown that the abundance of nematodes and rotifers is significantly increased by fertilizers; rotifers were stimulated to a similar extent by biosol and NPK, while bactosol (formerly named ARA) had much less effect (Lüftenegger et al. 1986). The opposite applied to the nematodes; thus, as in our study, dried bacterial biomass (bactosol) was superior to other fertilizers with respect to this group of soil animals. It has also been reported that thomasphosphate did not affect the nematodes in an alpine pasture, while their number was strongly reduced by NPK and lime (Berger et al. 1986).

## Discussion

The generally increased values of most biological parameters in the treated soil strongly suggest that the fertilizers applied did not harm the micro-edaphon. However, individual numbers and catalase activities were strongly reduced in September 1986 (Fig. 1b–h). This may be ascribed to the destruction of the natural topsoil by the planting procedure and the dilution effect of the "paper pot soil" (see Site description). The ciliates and testaceans recovered, i.e., approached the abundance expected at this altitude (Foissner et al. 1982; Foissner 1985; Berger et al. 1986; Lüftenegger et al. 1986), within 1 year, but the nematodes and rotifers needed about 2 years (Fig. 1c, e, h). Recovery by the ciliates and nematodes was accelerated by adding bacterial biomass, and 3 months after planting their numbers were considerably higher in these sites (CDEFIKL) than in the control site (A; Fig. 1f, h). Similar results were obtained in soils on revegetated ski slopes (Lüftenegger et al. 1986).

Two years after applying the total amount of fertilizer, soil life was still stimulated compared to the control (A), but the values clearly tended to converge (Fig. 1b–h). The fertilizer effects were thus not sustainable; the greatest differences were observed between 1988 and 1989, 1 year after refertilization. In a similar study of plant growth, Köck and Holaus (1981) observed the same trend. They concluded that repeated annual organic fertilizer applications would be needed for a lasting restoration of ski slopes.

The most pronounced soil life stimulation in the present study occurred in treatments with 180–270 g organic material (EFL). Higher quantities of bactosol with magnesite (L) changed the soil life to a similar extent as pure bactosol (EF), although pH values differed by about 1.5 units (Fig. 2b, d, f; Tables 3, 4). This indicates that the organic and nutrient quantity applied (180 g per seedling as in treatment E) was apparently more decisive than the pH level (cf. Foissner 1987b). This interpretation is supported by the observation that the other magnesite-dominated treatments (GHIK; Fig. 2b, d, f), which raised the soil pH, had much weaker effects; these treatments, however, contained much less organic material than the bactosol treatments (EFL). In addition, the 54–108 g organic material applied in treatments CD was less effective than the higher amounts side-dressed in treatments EF. No substantial differences were found between the biomag (G) and the NPK and magnesite treatments (H) in pH and ciliate numbers, while catalase activity and testacean numbers were slightly decreased by the NPK combination (H). Moreover, in view of the shorter treatment period and the smaller amount applied in the biomag treatment (G), this fertilizer seems preferable to the NPK and magnesite combination (H). The NPK fertilizer (B) had almost no effect on the pH and the biological parameters.

In their capacity to stimulate soil life, the fertilizers can be ranked as follows (Fig. 1b–h; Table 5): Bactosol (CDEF) > bactosol and magnesite (IKL) > biomag (G) > NPK and magnesite (H) > NPK (B). This ranking was confirmed by analyses of the testacean and ciliate community structure, i.e., the organic fertilizers caused greater changes than the organomineral ones (Fig. 2a–f). Our conclusion that organic fertilizers are more effective agrees with results reported by Köck and Holaus (1981), Insam and Haselwandter (1985), Lüftenegger et al. (1986), and Kilian and Glatzel (1989), who studied the revegetation of ski slopes and the revitalization of alpine woodlands. The increased numbers of animals in the organically fertilized treatments possibly improved nutrient availability, through bacterial and fungal grazing (Beck 1989). Our results and the ranking of the fertilizers are largely in agreement with the silvicultural investigations of the present reforestation trial, e.g., increased growth and a more healthy appearance of the organically grown spruce seedlings (Glatzel et al. 1991).

*Note.* Trade names and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the products by the University of Salzburg.

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