

## Changes in microbial nutrient status during secondary succession and its modification by earthworms

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**Summary.** Microbial biomass, nutrient (N and P) status, and carbon and nutrient limitation of the microflora were investigated in soils from five different sites (field, 5-, 12-, and about 50-year-old fallow, beechwood), which represent different stages of a secondary succession from a wheat field to the climax ecosystem of a beechwood on limestone. In addition, the effect of faeces production by the substrate feeding earthworm species *Octolasion lacteum* (Örley) on the nutrient status of the soil microflora of these sites was studied. Humus had accumulated in the soil of the third fallow site, with an enhanced biomass of microflora. However, in the beechwood soil, which had the highest humus content, microbial biomass was lower than in the soil of the third fallow site and similar to that of the field and the two younger fallow sites. In general, soil microbial biomass was little affected by the passage of soil through the gut of *O. lacteum*. The soil microflora of the field, the 5-, 12-, and about 50-year-old fallow was limited by carbon, whereas in the beechwood soil phosphorus limited microbial growth. Nitrogen availability to the soil microflora was low in the two younger fallow sites and high in the field and the third fallow. In the beechwood soil nitrogen supply did not affect microbial carbon utilization. Application of phosphorus stimulated glucose mineralization in the soil of the field, the third fallow, and the beechwood, but not in the two younger fallow sites. Therefore, the nutrient status of the soil microflora seems to have changed during secondary succession: presumably, during the first phase the availability of nitrogen decreased, whereas during the second phase microbial phosphorus supply became more important, which resulted in phosphorus limitation of the soil microflora in the climax ecosystem. The passage of soil through the gut of *O. lacteum* caused an alteration in the microbial nutrient status. Generally, microbial growth in earthworm casts was limited by carbon. The relative effect of the gut passage of the soils on microbial carbon utilization seems to increase during succession. Therefore, the effect of decomposer invertebrates on microbial nutrient supply seems to increase during secondary suc-

cession. In general, nitrogen did not limit microbial carbon utilization in earthworm casts. Phosphorus requirements of the soil microflora were lowered by the gut passage of the soil of the third fallow site and the beechwood, which indicates an increased phosphorus supply in earthworm casts. However, this additional supply was not sufficient to enable optimal carbon utilization by the soil microflora. The results indicate that the effect of decomposer invertebrates on the soil microflora depends on the nutrient status of the ecosystem.

**Key words:** Microbial nutrient status – Microbial nutrient limitation – Microbial biomass – Secondary succession – Earthworms

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An essential process during the development of terrestrial ecosystems (primary succession) is the immobilization of nitrogen and the accumulation of humus (e.g. Reiners 1981). In contrast, during secondary succession following clear-cutting of woods excess nitrogen mineralization occurs, which results in a nitrogen loss from the system (e.g. Vitousek 1981). A different pattern of changes in nutrient mineralization (particularly that of nitrogen) is found during secondary succession of abandoned fields. In fallow sites the nutrient supply to plants often decreases with time (e.g. Odum 1960; Golley and Gentry 1965; Hahn et al. 1979; Wiegert and McGinnis 1975). To elucidate changes in the nutrient status of the soil microflora during secondary succession, this study investigated five different sites, which represent different stages of secondary succession from a wheat field to the climax ecosystem of a beechwood on limestone. Glucose and fertilizers (nitrogen and phosphorus) were added to the soil of these sites to measure carbon limitation and nutrient deficiencies of the soil microorganisms.

Nutrient immobilization and mineralization is mainly caused by the soil microflora; however, the microbial nutrient turnover is considered to be driven by the activity of the soil fauna (e.g. Coleman et al. 1983; Anderson

1987). Studies in the laboratory on the effect of soil invertebrates on microbial nutrient cycling should focus on animals, which may be of considerable importance in this process in the field. One of the most important groups of decomposer invertebrates of the areas studied is lumbricids (Scheu in preparation). Therefore, the effect of faeces production by lumbricids on the nutrient status of the soil microflora of the study sites was investigated. Of the earthworm species present at the study sites the substrate feeding *Octolasion lacteum* (Örley) was chosen for the experiments, because substrate feeding species are considered to be especially important for soil turnover (Scheu 1987a) and may therefore strongly affect the environment of the soil microflora. To study the effect of earthworms on the microbial nutrient demands, glucose and different fertilizers were also added to casts of *O. lacteum* deposited in the soil of the study sites.

This experiment enabled a closer understanding of the changes in microbial nutrient status during a succession from a field depending on external nutrient applications to a climax ecosystem with internal nutrient turnover, and their modification by the activity of earthworms.

### The sites

Five different sites were studied, which represent different stages of secondary succession from a sown wheat field to a beechwood on limestone. All sites were located on the limestone plateau (360–420 m) east of Göttingen (Northern Germany). The first site was a field, which had been planted for 2 years with wheat. The second site was a fallow, which had been left uncultivated for 5 years ('first fallow'). The flora consisted of a mixture of weeds (e.g. *Matricaria inodora* L., *Geranium dissectum* Jusl., *Myosotis arvensis* (L.)), species of ruderal sites (e.g. *Epilobium montanum* L., *Tussilago farfara* L., *Reseda luteola* L.) and pastures (e.g. *Phleum pratense* L., *Dactylis glomerata* L.). The third site ('second fallow') was an abandoned field, which had been left uncultivated for 12 years. Grasses (e.g. *Dactylis glomerata* L., *Poa angustifolia* L., *Arrhenatherum elatius* (L.), *Trisetum flavescens* (L.), *Poa pratensis* L.) dominated at that site. Some species typical of pastures with low nutrient supply were also present (e.g. *Rhinanthus serotinus* (Schönh.), *Pimpinella saxifraga* L.) and several shrubs (mainly *Rosa* sp.) had grown up to about 2 m. The fourth site ('third fallow') had been left uncultivated for more than 50 years and was now dominated by ash (*Fraxinus excelsior* L.). The fifth site was an about 130-year-old mull beechwood, which has been described in detail by Schaefer (1990). The soils of the study sites were of the Rendzina type, more or less shallow and to some extent containing loess.

### Materials and methods

Soil samples of about 50 × 50 cm were taken from the upper 3 cm of the mineral soil of the five study sites after removal of the litter layer, and passed through a 4 mm sieve. Before sieving plant roots were carefully excluded by hand. The soil was placed in

cages in which the burrowing activity of earthworms could be observed. They consisted of two perspex sheets (190 × 150 × 3 mm), separated by plastic strips (10 mm wide and 3 mm thick; cf. Evans 1947; Scheu 1987a). One specimen of the substrate feeding earthworm species *O. lacteum* was added to each of half of the cages. The earthworms were sampled by hand in the beechwood immediately before the experiments were set up. After the cages had been incubated for 6 days at 15° C in permanent darkness the earthworm casts were separated carefully from the soil by means of two fine spatulas. Faeces particles deposited during the whole incubation period of 6 days were collected. Earthworm casts and soil from untreated cages were used as substrates for the experiment. Fresh substrates equivalent to 0.7 g dry weight were placed in glass vessels of about 12 ml volume immediately after sampling.

Experiments were set up in June 1987 to study the microbial nutrient status during intensive plant growth, i.e. during nutrient competition between the soil microflora and plants.

Microbial biomass was measured by the method of Anderson and Domsch (1978), using the level of microbial respiration reached 0–6 h after the addition of glucose ('initial response') to calculate the microbial biomass in the soil. Glucose (and fertilizer) was added in aqueous solutions. The amount of solutions applied increased the water content of the substrates to 1 ml g<sup>-1</sup> dry weight. Microbial respiration was measured manometrically by a Warburg respirometer (Braun Company, Melsungen) at 22° C, so respiration was determined by O<sub>2</sub> consumption of the microflora. This is preferable to measuring CO<sub>2</sub> production especially in soils rich in carbonate. To calculate microbial biomass a respiratory quotient of 1 was used, which is characteristic for the oxidation of carbohydrates like glucose.

The nutrient limitations and deficiencies of the soil microflora were investigated by the addition of glucose and different fertilizers to the soil and casts from the five study sites (three replicates per treatment):

- (1) Glucose (C)
- (2) Glucose and nitrogen (CN)
- (3) Glucose and phosphorus (CP)
- (4) Glucose, nitrogen, and phosphorus (CNP).

8000 mg of glucose kg<sup>-1</sup> dry weight were added to the soil. Since for the soil microflora a ratio between C, N, and P of 10:2:1 is assumed (cf. Anderson and Domsch 1980) this ratio was used in the fertilization treatments.

The cumulative additional respiration of the soil microflora (following the 'initial response') up to the maximum respiration rate (after 25–45 h) was measured. For a detailed record of the time course, microbial respiration was usually measured every hour.

The additional respiration following the 'initial response' is caused by increased energy metabolism of the growing microflora. Therefore, the additional microbial respiration can be used to estimate the ability of the microflora to grow, i.e. to increase their biomass. Microbial growth depends on the availability of nutrients, hence the increase in respiration caused by the addition of different nutrients is a good measure of the nutrient demands of the soil microflora for these nutrients (Vanselow in prep.).

Carbon and nitrogen content of the soils and earthworm casts were determined by means of an elemental analyser (Carlo Erba Company, Milano).

The data were analysed by a four-way analysis of variance. Factors were substrate (soil and faeces), soil type (field, first, second, and third fallow site, beechwood), nitrate fertilization, and phosphorus fertilization. The data were log-transformed prior to analysis. Differences between means were inspected using Tukey's honestly significant difference at the 0.05 level (Sokal and Rohlf 1981).

### Results

#### *Carbon and nitrogen contents*

The carbon content of the soil of the field, the first, and the second fallow were similar and distinctly lower

**Table 1.** Carbon and nitrogen content and C/N ratio of the soil of the five study sites and of casts of *Octolasion lacteum* deposited in these soils (means of three replicates with standard deviations)

	C content (%)				N content (%)				C/N ratio	
	Soil		Casts		Soil		Casts		Soil	Casts
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	Mean
Field	3.25	0.13	3.19	0.16	0.31	0.01	0.30	0.01	10.3	10.6
First fallow	3.83	0.14	3.72	0.14	0.29	0.02	0.28	0.01	13.2	13.3
Second fallow	3.26	0.06	3.36	0.17	0.24	0.01	0.24	0.01	13.6	14.0
Third fallow	7.04	0.14	6.94	0.16	0.51	0.02	0.49	0.02	13.8	14.2
Beechwood	8.25	0.16	8.05	0.12	0.56	0.01	0.54	0.01	14.7	14.9

**Table 2.** Microbial biomass of the soil of the five study sites in June 1987 and of casts of *Octolasion lacteum* deposited after ingestion of soil of these sites (means of three replicates; values sharing the same superscripts are not significantly different at the  $P < 0.05$  level, Tukey's honestly significant difference)

	Microbial biomass ( $\mu\text{gC g}^{-1}$ dry weight)				
	Field	First fallow	Second fallow	Third fallow	Beechwood
Soil	1535 <sup>b, c</sup>	1366 <sup>a, b, c</sup>	1268 <sup>a, b</sup>	3532 <sup>d</sup>	1171 <sup>a</sup>
Casts	1201 <sup>a</sup>	1582 <sup>c</sup>	1317 <sup>a, b, c</sup>	3418 <sup>d</sup>	1214 <sup>a</sup>

than that of the third fallow and the beechwood (Table 1). In addition, the soil nitrogen content of the third fallow and the beechwood exceeded that of the younger fallow sites and the field considerably. Therefore, humus seems to have accumulated during secondary succession.

The carbon and nitrogen content and the C/N ratio of earthworm casts deposited in the soil of the study sites were almost identical to that of the uningested soil (Table 1). This indicates that the earthworms ingested the soil indiscriminately and did not select organic particles.

#### Microbial biomass

Microbial biomass of the soil of the study sites was in the range of 1171–1535  $\mu\text{gC g}^{-1}$  dry weight with the exception of the third fallow (Table 2). In general, the biomass of the microflora was little affected by the gut passage of the soil. The microbial biomass of the field soil was the only one reduced significantly by passage through the gut of *O. lacteum* (–22%; Table 2). In comparison to the soil of the other sites both the soil of the third fallow site and casts deposited in that soil had an almost threefold microbial biomass.

#### Fertilizer treatments

An overall 99.7% of the variation in additional respiration could be explained by the treatments (Table 3). Of

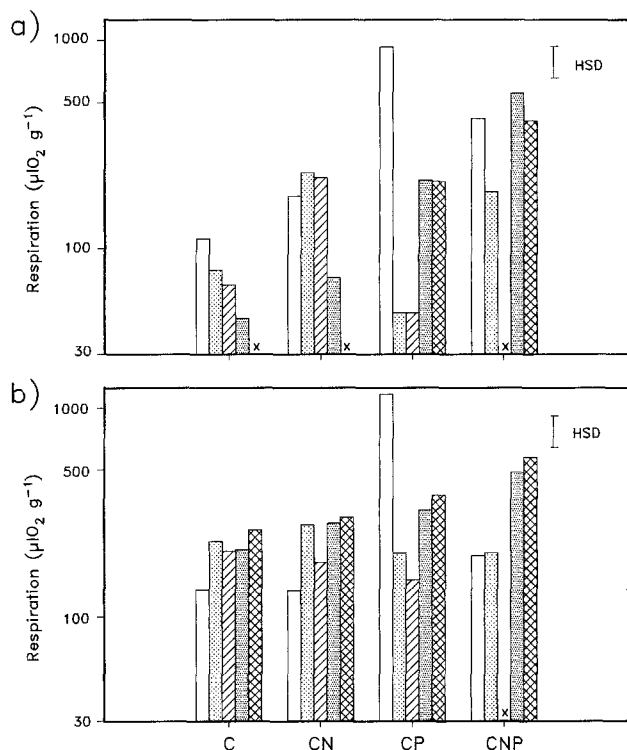
**Table 3.** Four-way analysis of variance with log-transformed data of the additional microbial respiration caused by fertilizer applications. Factors were substrate (soil and casts), site (field, 5-, 12-, and about 50-year-old fallow, beechwood), nitrogen application, and phosphorus application (\*\*\*:  $P < 0.001$ )

Source of variation	SS	DF	F
Substrate	5.34	1	1033***
Site	11.74	4	2063***
Nitrogen	0.22	1	85***
Phosphorus	1.41	1	545***
Substrate $\times$ Site	6.70	4	997***
Substrate $\times$ Nitrogen	0.67	1	260***
Substrate $\times$ Phosphorus	2.36	1	910***
Site $\times$ Nitrogen	5.38	4	519***
Site $\times$ Phosphorus	21.81	4	2106***
Substrate $\times$ Site $\times$ Nitrogen	0.29	4	28***
Substrate $\times$ Site $\times$ Phosphorus	5.71	4	552***
Substrate $\times$ Nitrogen $\times$ Phosphorus	0.03	1	13***
Site $\times$ Nitrogen $\times$ Phosphorus	5.99	4	579***
Substrate $\times$ Site $\times$ Nitrogen $\times$ Phosphorus	0.02	4	2 n.s.
Residual	0.21	80	
Total	69.69	119	

the main effects, site had the most pronounced influence; 16.9% of the variation could be accounted for by this factor. The effect of phosphorus application depended strongly on study site. The interaction between both factors explained a further 31.3% of the variation. Of the three-way interactions, the interaction between site and phosphorus with the factors substrate and nitrogen accounted for an additional 8.2% and 8.6% of the variation. All main effects and the two way and three way interactions were highly significant ( $P < 0.001$ ).

#### Carbon limitation

The application of glucose induced microbial growth in the soil of all sites except that of the beechwood (Fig. 1 a). Therefore, in four of the five soils studied the microflora was limited by carbon. However, the ability to utilize additional carbon differed strongly between sites and decreased in the soil of late successional stages



**Fig. 1a, b.** Increase in microbial respiration caused by the addition of glucose (C), glucose and nitrogen (CN), glucose and phosphorus (CP) and glucose, nitrogen and phosphorus (CNP) (sum of additional microbial respiration following the "initial response" up to the maximum; mean of three replicates; HSD: Tukey's honestly significant difference,  $P < 0.05$ ; x: no increase in respiration). **a** Soil of the five study sites (field, first, second, and third fallow site, beechwood). **b** Casts of *Octolasion lacteum* deposited after ingestion of soil of the five study sites

(Fig. 1a). The decrease in carbon utilization ability of the soil microflora with the age of the fallow sites indicates an increasing nutrient deficiency of the microorganisms during succession. Therefore, the availability of nutrients seems to decrease during secondary succession.

In casts of *O. lacteum* from all sites the addition of glucose induced microbial growth (Fig. 1b). Hence, microbial growth in earthworm faeces produced in all soils studied had been limited by carbon. The lowest carbon utilization occurred in casts deposited after feeding on field soil, whereas the additional respiration of the microorganisms in casts deposited after ingestion of soil from the other four sites was significantly higher (Fig. 1b). The effect of the gut passage of the soil on the ability of the microflora to utilize additional carbon for growth differed between study sites. Microbial respiration in earthworm casts was increased in comparison to soil by the following factors: field 1.2, first fallow 2.8, second fallow 3.0, third fallow 4.5 and beechwood soil from 0 to 257  $\mu\text{O}_2 \text{ g}^{-1}$  dry weight. Thus, the relative effect of the animals seems to increase during succession. Presumably, the increased carbon utilization of the microflora in earthworm casts was caused by nutrients which were mineralized during the gut passage of the soil. The increase in the relative effect of the animals

with fallow age indicates a decrease in nutrient availability of the soil microflora during succession.

#### Nitrogen limitation

With the exception of the beechwood soil, additional fertilization with nitrogen caused a significant increase in the microbial carbon utilization ability in the soils (Fig. 1a). This indicates that in all these soils microbial utilization of carbon was limited by nitrogen. In comparison to application of glucose only, an additional nitrogen supply caused an increase in microbial respiration by factors of 1.6 (field), 2.9 (first fallow), 3.2 (second fallow) and 1.6 (third fallow). Therefore, the relative effect of nitrogen increased up to the second fallow stage. The amount of additional microbial respiration in soil of the third fallow site caused by CN fertilization was significantly lower than that in the soil of the younger fallow sites and the field (Fig. 1a). This indicates that the nutrient status of the microflora of the third fallow site was intermediate between that of the beechwood soil (no microbial growth stimulated by C and N fertilization) and that of the soil of the younger fallow sites and the field (greater microbial growth stimulated by C and N addition).

In earthworm casts additional fertilization with nitrogen did not increase microbial respiration (Fig. 1b). The amount of nitrogen available in casts was apparently sufficient to enable the microflora to utilize additional carbon. Therefore, nitrogen did not limit microbial growth in faeces of earthworms. Microbial respiration in glucose-supplemented casts was similar to that in CN-fertilized soil in the case of the field and the two younger fallow sites (Fig. 1). This indicates that the effect of the gut passage of these soils on microbial carbon utilization ability was caused exclusively by enhanced nitrogen availability in the casts. In contrast, the effect of the gut passage of the soil of the third fallow and that of the beechwood could not be explained sufficiently by increased nitrogen availability in casts, because microbial respiration of C-fertilized casts exceeded that of CN-fertilized soil significantly (third fallow soil by a factor of 2.9, beechwood soil 257 to 0  $\mu\text{O}_2 \text{ g}^{-1}$  dry weight; Fig. 1b). This conclusion can also be drawn by a comparison of CN-fertilized soils and casts: the gut passage of the soil of the third fallow site and that of the beechwood caused a significant increase in microbial respiration (by a factor of 3.9 and from 0 to 302  $\mu\text{O}_2 \text{ g}^{-1}$  dry weight respectively).

#### Phosphorus limitation

The response of the microflora of the five soils to an additional supply of phosphorus differed strongly. In the beechwood soil the microflora was enabled to utilize the carbon supply provided, whereas an addition of glucose only or glucose and nitrogen did not result in an increase in respiration (Fig. 1a). In the soil of the third fallow and the field microbial respiration was also in-

creased by additional fertilization with phosphorus (by a factor of 4.7 and 8.3 respectively). However, in the first and second fallow fertilization with phosphorus had no effect or even decreased microbial respiration in comparison to the control (Fig. 1a). In the soil of the field and the third fallow microbial respiration was increased more strongly by addition of phosphorus than by addition of nitrogen, whereas in the soil of the first and second fallow a supply of nitrogen had the stronger effect.

In earthworm casts deposited in the soil of the field, the third fallow site, and the beechwood, additional application of phosphorus caused an increase in microbial respiration in comparison to glucose application (by a factor of 8.7 ( $P < 0.05$ ), 1.6 ( $P < 0.05$ ) and 1.4 (n.s.) respectively; Fig. 1b). In contrast, additional P supply had no effect in casts produced in the soil of the first and second fallow (Fig. 1b). Microbial respiration in both casts and soil of the field fertilized by phosphorus was by far the highest of all treatments (1175 and 933  $\mu\text{O}_2 \text{ g}^{-1}$  dry weight; Fig. 1b). Therefore, carbon utilization of the field microflora was most effective.

Addition of phosphorus to the soil of the third fallow and the beechwood resulted in a dramatic increase in microbial respiration in comparison to the control, whereas the effect in casts of these soils was much lower (Fig. 1b). This suggests that the observed much higher respiration in glucose-supplemented casts of the third fallow and the beechwood in comparison to the respective soils was caused by an enhanced phosphorus supply in the casts. However, the greater phosphorus availability in earthworm casts in comparison to the soil was not sufficient to enable optimal carbon utilization by the microflora.

#### *Fertilization with glucose, nitrogen, and phosphorus*

In the beechwood soil and that of the third fallow site CNP application caused the strongest increase in microbial respiration (Fig. 1a) indicating a strong interaction of nitrogen and phosphorus. In comparison to CN fertilization of these soils microbial respiration was enhanced from 0 to 398  $\mu\text{O}_2 \text{ g}^{-1}$  dry weight and by a factor of 7.8 ( $P < 0.05$ ) respectively. In comparison to CP fertilization it was increased by a factor of 1.5 ( $P < 0.05$ ) and 2.6 ( $P < 0.05$ ). Therefore, in both soils microbial growth of the CN-fertilized microflora was limited by phosphorus and that of the CP-fertilized microflora by nitrogen. However, in both soils the effect of additional phosphorus exceeded that of nitrogen considerably.

In the field soil CNP fertilization caused an increase in microbial respiration in comparison to CN application (by a factor of 2.3 ( $P < 0.05$ )), whereas in comparison to CP application glucose mineralization was decreased ( $-57\%$  ( $P < 0.05$ ); Fig. 1a). In the soil of the first fallow an additional application of phosphorus had no significant effect on microbial respiration in comparison to CN fertilization only (Fig. 1a). In contrast, CNP fertilization of this soil increased carbon mineralization in comparison to CP application by a factor of 3.8 ( $P <$

0.05). As was already shown, only nitrogen limited microbial carbon utilization in this soil. In the soil of the second fallow CNP fertilization resulted in a dramatic reduction in microbial respiration. The respiration rates even dropped considerably below the level of the "initial response" in C-fertilized soil.

Generally, in earthworm casts the effects of CNP fertilization on microbial respiration were similar to those in the respective soils. However, in comparison to CN fertilization, CNP application caused a much lower increase in microbial respiration in casts of the third fallow and the beechwood than in the soils of these sites. Presumably, this was caused by higher phosphorus availability in earthworm faeces. CNP application to casts deposited in the soil of the third fallow and the beechwood caused a similar increase in microbial respiration in comparison to both CN and CP fertilization (Fig. 1b).

#### **Discussion**

During the development of terrestrial ecosystems an accumulation of carbon and nutrients occurs, which results in the formation of a complex decomposer community. Carbon mineralization and nutrient recycling are mainly due to the soil microflora. However, soil animals modify the microbial environment considerably and may affect therefore the microbial carbon turnover and nutrient recycling (cf. Coleman et al. 1983; Anderson and Ineson 1984). Little is known about the influence of the accumulation of carbon and nutrients in dead tissue and humus material during succession on the nutrient status of the microflora and its modification by the activity of decomposer invertebrates.

Studies on the secondary succession of terrestrial ecosystems up to climax are for practical reasons usually performed at different sites, which are considered to represent different stages of succession. This approach involves the difficulty that the observed coexistence in space does not necessarily represent the real time course of succession. The sites investigated in this study were carefully chosen to minimize this difficulty. They were located close together, had a similar history, and were on almost the same soil type.

Several authors have found an enhanced microbial biomass in soils richer in carbon (Schnürer et al. 1985; Insam and Domsch 1988; Diaz-Ravina et al. 1988). The soil carbon and nitrogen content of the sites investigated in the present study was higher in the late successional stage and in the climax ecosystem. However, the enhanced humus content of these sites was not accompanied by an equivalent increase in microbial biomass. The beechwood soil had the highest humus content, but contained less microbial biomass than the four other soils studied. Microbial biomass of the soil of the third fallow site, in which considerable humus accumulation had occurred, was extraordinarily high and exceeded that of the other sites by a factor of about 3. Therefore, no correlation was found between the formation of soils rich in humus and microbial biomass.

The ratio between microbial biomass and the organic content of soils has been used as a measure of the availability of the organic substrate of soils (Insam and Domsch 1988). The ratio is considered to reflect the degree of stability of humus material, which is assumed to increase during ecosystem development. In the field soil the ratio was higher (0.047) than in the soil of the younger fallow sites (0.036 and 0.039), and in soil of the third fallow (0.050) it was similar to that in field soil. Therefore, this ratio did not reflect the accumulation and increased stability of humus material in soil of the third fallow site. In the beechwood soil the ratio was very low (0.014) and indicates the high stability of humus material of that soil. In general, the usefulness of this ratio is considered to be limited.

The nutrient status of the soil microflora of the five study sites was investigated in June. At the end of spring plant growth is intensive at all sites studied. Therefore, the nutrient status of the soil microflora of the study sites that was found is assumed to reflect the nutrient status of the five ecosystems during the season when the nutrient characteristics of these ecosystems are most pronounced.

The nutrient deficiency of the soil microflora was greater at sites representing later stages of secondary succession. In addition, the element limiting microbial growth seems to have changed during succession (Fig. 2a). Carbon limited microbial growth in the soil of the field and the 5-, 12-, and about 50-year-old fallow sites, but as already had been shown previously (Vanselow pers. comm.; Domsch 1982; Scheu 1987b) the microflora of this beechwood soil was limited by phosphorus.

The nitrogen deficiency of the soil microflora increased till the stage of the second fallow site. This indicates that during the first phase of secondary succession the availability of nitrogen decreases. This conclusion can also be drawn by comparing the flora of the sites in more detail. Up to the stage of the second fallow, plants indicative of lower nitrogen supply became more abundant (Scheu 1990). A decrease in nitrogen supply to plants with fallow age has been reported by several authors (Odum 1960; Golley and Gentry 1965; Hahn et al. 1979; Wiegert and McGinnis 1975). However, with the establishment of trees (mainly ash) in the third fallow in the present study the demand of the soil microflora for phosphorus considerably exceeded that for nitrogen. Therefore, the nutrient status of the microflora of this site is assumed to be intermediate between that of the beechwood, in which microbial growth was limited by phosphorus and that of the younger fallow sites, in which the microflora had high nitrogen and no phosphorus demands (see Fig. 2a).

The most pronounced increase in microbial respiration occurred in casts and soil of the field (CP fertilization). Therefore, the soil microflora of the field was by far the most effective in carbon mineralization and growth. The lower carbon utilization of the soil microflora of the other sites may be caused by a lack of nutrients other than nitrogen and phosphorus. However, in comparison to earthworm casts deposited in field soil,

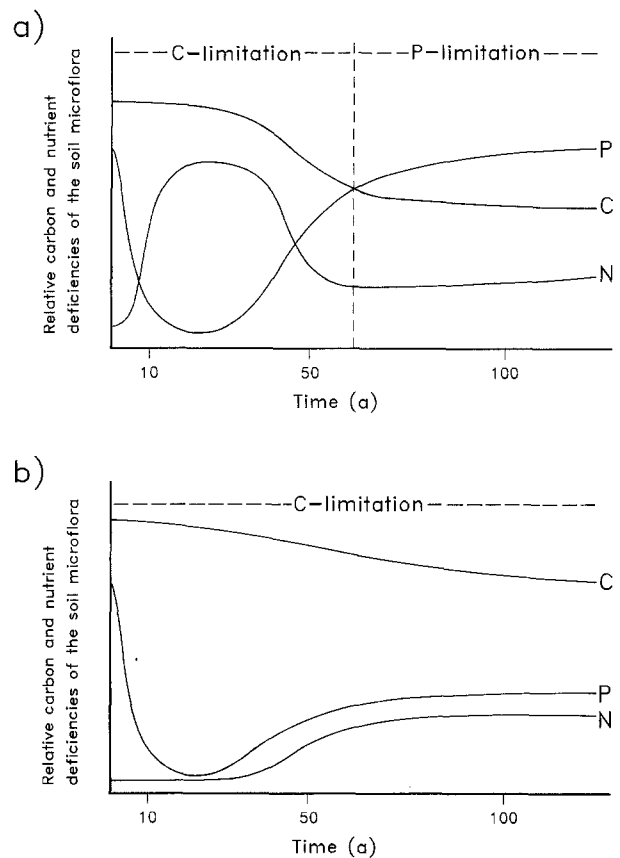


Fig. 2a, b. Scheme of changes in the relative carbon and nutrient deficiencies of the soil microflora during secondary succession from a field to a beechwood (for details see text) a Soil b Casts of *Octolasion lacteum* deposited in these soils

carbon utilization ability of the microflora of casts produced in soil of the four other sites was also distinctly lower. Earthworm casts are known to contain greater amounts of several nutrients (e.g. Graff 1971; Czerwinski et al. 1974). Therefore, the lower ability to increase microbial growth in soil of the other four sites in comparison to the field may be caused by slow and ineffective resource allocation of the microflora of these sites. The lowest carbon utilization and therefore microbial growth occurred in the soil of the two younger fallow sites. This may be related to low nutrient turnover in these ecosystems. In contrast, the microflora of the field may be adapted to sudden and high nutrient fluctuations.

In comparison to CN fertilization, the additional application of phosphorus to field soil caused a reduction in microbial oxygen consumption during growth. This may have been caused by an increased use of nitrate (from the added ammonium nitrate) by the microflora as an alternative electron acceptor, which could be of considerable importance even under aerobic conditions (Ottow and Fabig 1984). However, it remains unexplained why the additional fertilization with phosphorus accelerated this process. The use of nitrate as an alternative electron acceptor may also be responsible for the drop in microbial oxygen consumption in CNP-fertilized soil of the second fallow and the respective earthworm

casts. Phosphorus was also responsible for the induction of this process, because it did not occur in CN-fertilized soil. Presumably, the addition of phosphorus favoured the growth of microorganisms which could use nitrate as an alternative electron acceptor. In comparison to C application, additional fertilization with phosphorus of the soil of the two younger fallow areas caused a significant reduction in microbial oxygen consumption. The reason for this phenomenon remains unclear, because mineral nitrogen (and therefore nitrate) availability was low in these soils.

Microbial growth in earthworm casts deposited after ingestion of all soils studied was limited by carbon (Fig. 2b). Presumably, the elimination of phosphorus limitation in beechwood soil and the reduction in phosphorus deficiency of the soil microflora of the third fallow site by the gut passage of the soil was caused by an increased phosphorus availability in earthworm casts. An enhanced amount of phosphate in earthworm faeces has often been reported (Graff 1971; Aldag and Graff 1975; Sharpley and Syers 1977; Mansell et al. 1981). However, in casts deposited in field soil the phosphorus deficiency of the microflora was similar to that of uningested soil. Presumably, the additional phosphate in casts produced in soil was immobilized quickly. Immobilization of phosphate has been shown to occur in ageing earthworm faeces (Scheu 1987b).

Microbial carbon utilization and growth in earthworm casts was not limited by nitrogen. Presumably, additional mineral nitrogen available in earthworm casts (cf. Lee 1985; Scheu 1987c) was sufficient to enable optimal carbon utilization by the microflora. However, microbial oxygen consumption of casts produced in soil of the third fallow site and the beechwood fertilized with carbon and phosphorus could be increased by additional application of nitrogen (ammonium nitrate). Increased oxygen consumption caused by nitrification of the added ammonium may have been responsible for this process. The number of nitrifying bacteria is known to be increased in earthworm faeces (Loquet et al. 1977), and therefore nitrification may be accelerated in casts. In previous experiments it was shown that oxygen consumption of earthworm casts could be enhanced by additional fertilization with ammonium but not with nitrate (Scheu 1987b).

The limitation of microbial growth in earthworm casts indicates that the ingestion of additional carbon resources would cause an increase in microbial biomass in casts. Scheu and Sprengel (1989) showed that feeding on and removal of faecal pellets of the millipede *Glomeris marginata* by the substrate feeding earthworm *Aporrectodea caliginosa* resulted in an increased microbial biomass in the soil. Due to selective feeding, earthworm casts are usually richer in carbon than the surrounding soil (cf. Lee 1985). Therefore, an increase in microbial biomass of the soil due to the activity of earthworms is considered to be a common phenomenon, which maintains the long-term nutrient supply of the microflora of the mineral soil.

In conclusion, in the soil of the third fallow and of the beechwood the increased availability of phospho-

rus in earthworm casts was most important for microbial growth. In contrast, in the soil of the younger fallow sites an enhanced nitrogen availability in earthworm faeces was more important (see Fig. 2a, b). Therefore, the effect of an additional nutrient supply in earthworm casts depends on the nutrient status of the soil microflora. Presumably, during the first phase of secondary succession faeces production of earthworms mostly affects the nitrogen supply of the soil microflora, whereas in late successional stages and in climax ecosystems the effect of earthworm activity on microbial phosphorus supply is more important. Phosphorus limitation of microbial growth combined with high activity of soil invertebrates (Schaefer 1990) may be a key component ensuring the high productivity of the beechwood studied. Presumably, animal activity is more important for the cycling of nutrients which have mineral pools with high turnover rates, like phosphorus. The necessity of a more intensive consideration of the time scale in studies on nutrient cycling and animal activity has recently been stressed (Anderson 1988).

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