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DOI

[10.1007/s00374-009-0354-2](https://doi.org/10.1007/s00374-009-0354-2)

Publication date

2009

Document Version

Final published version

Published in

Biology and Fertility of Soils

[Link to publication](#)

Citation for published version (APA):

Kooijman, A. M., van Mourik, J. M., & Schilder, M. L. M. (2009). The relationship between N mineralization or microbial biomass N with micromorphological properties in beech forest soils with different texture and pH. *Biology and Fertility of Soils*, 45(5), 449-459. <https://doi.org/10.1007/s00374-009-0354-2>

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The relationship between N mineralization or microbial biomass N with micromorphological properties in beech forest soils with different texture and pH

A. M. Kooijman · J. M. van Mourik · M. L. M. Schilder

Received: 24 July 2008 / Revised: 31 December 2008 / Accepted: 8 January 2009 / Published online: 27 January 2009
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Abstract To test relationships between net N-mineralization, organic matter and soil organisms, we combined micromorphology with laboratory incubation experiments over a soil gradient. Microbial biomass N generally increased with pH, and from sandy to loamy soil, but net N-mineralization showed the opposite, and was highest in acid, sandy soil. Twenty-two micromorphological characteristics were analyzed with principal component analysis. PC1 had high eigenvalue (0.70), and clearly separated fungi from earthworms, microarthropods and bacteria. PC2 was less important (0.15). Organic layer and sand content clearly correlated with the fungi-end of PC1, but pH and C-content of the Ah with the opposite. Microbial N also correlated with the earthworm–bacteria end, but net N-mineralization did not. Efficiency of N-mineralization per unit microbe even correlated with the fungi end of PC1, in both organic layer and mineral topsoil. The results support the hypothesis that high (or low) litter turnover and biological activity can be counteracted by high (or low) microbial N-demand.

Keywords Bacteria · *Fagus sylvatica* L. · Fungi · Humus forms · Luxembourg · Net N-mineralization · Principal component analysis

Introduction

Soil conditions such as pH and texture are important factors regulating C and N dynamics, and lead to substantial

differences in litter decay, humus form, and N-release (Swift et al. 1979; Green et al. 1993; Ponge 2003). Acid and/or sandy soils have low rates of decomposition, low depolymerization of N-containing polymers by microbial enzymes, and presumably also low net N-release to the vegetation (e.g., Aerts and Chapin 2000; Ponge 2003; Schimel and Bennett 2004). In contrast, at high pH, or in loamy soil, net N-mineralization is supposedly high, due to high rates of litter decay. However, in many field and laboratory studies, net N-mineralization showed a contradictory response (Zöttl 1960; Davy and Taylor 1974; Verhoeven et al. 1988, 1990; Hassink et al. 1993; Hassink 1994; Kooijman and Besse 2002), with high instead of low values in acid or sandy soils with low litter decay. In Kooijman et al. (2008), such unexpectedly high net N-mineralization was attributed to low microbial N-requirements, which could be related to the presence of fungi, which generally dominate acid soils (Blagodatskaya and Anderson 1998; Bååth and Anderson 2003). In calcareous soil, where bacteria are generally more abundant, gross N-mineralization was higher than in acid soil indeed, but net N-mineralization was reduced by high microbial N-demand.

The objective of this study was to further unravel relationships between net N-mineralization and soil organisms over soil gradients in pH and texture. Soil organisms can be studied in many different ways (e.g., Hågvar 1990; Scheu 1997; Blagodatskaya and Anderson 1998; Bååth and Anderson 2003; Moore et al. 2005; Marhan and Scheu 2005), but micromorphological analysis of thin sections offers a more integrative approach (van Mourik 2003; Davidson et al. 2004; Kapur et al. 2008). Soil fauna, microorganisms, and gradual changes in soil organic matter can be studied at the same time. Micromorphology has traditionally been rather qualitative and descriptive. We tried to circumvent that disadvantage by using a semiquantitative approach, with

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classification of 22 micromorphological characteristics on a scale of 0–3, varying from not detected to abundant, and multivariate analysis of all of them with principal component analysis. We selected six beech forests in Luxembourg: three on sandy soil and three on loamy, each with low, intermediate, and high pH. In each forest, we measured microbial N in the field, and net N-mineralization in laboratory incubation experiments in spring and autumn. To link changes in microbial N and net N-mineralization over this soil gradient to soil organisms such as earthworms, microarthropods, bacteria, and fungi, we studied thin sections of the upper soil. Because bacteria are more difficult to observe than fungi in sections, bacteria were also measured with plate cultures. Research questions were (1) How do microbial N and net N-mineralization in organic layer and mineral topsoil change along the soil gradients? (2) How do micromorphological characteristics change along the soil gradients? (3) How is N-cycling related to soil organisms?

Materials and methods

Selection of study sites

Six mature, monospecific beech (*Fagus sylvatica* L.) stands were selected on soils with different texture and pH in the area south of Diekirch, Luxembourg. At present, diameter at breast height (DBH) of most trees is more than 50 cm. Litter input from beech leaves did not differ between sites and ranged from 449 to 518 g m⁻² year⁻¹. The three forests on sandy soil (S1, S2, and S3) were located on Mesozoic sandstone in various stages of decalcification; S1 was

located on a plateau, had loamy sand texture and low pH (Table 1), an Ah-E-Bw-C soil profile with signs of recent podzolization, and forest type was *Fago-Quercetum* (van der Werf 1991); S2 was located near the edge of the sandstone plateau, had loamy sand texture and intermediate pH, Ah-Bw-C soil profile, and *Milio-Fagetum* forest type; S3 was located on former pit deposits in calcareous sandstone, had loamy sand texture and high pH, Ah-C soil profile, and *Melico-Fagetum* forest type. The three forests with loamy soil (L1, L2, and L3) were located on Tertiary river terrace and decalcified and calcareous Mesozoic marl; L1 had loam texture and low pH, Ah-E-Bt-C soil profile, and *Fago-Quercetum* to *Milio-Fagetum* forest type; L2 had silt loam texture and intermediate pH, Ah-E-Bt-C soil profile and *Melico-Fagetum* forest type, grading into *Stellario-Carpinetum*; L3 had clay loam texture and high pH, Ah-Bw-C soil profile, and *Carici-Fagetum* forest type. Cover of the understorey was generally (very) low, except in S2 and S3, which had large patches of *Melica uniflora* Retz. and/or *Mercurialis perennis* L. The four sites with low undergrowth were earlier used in Kooijman et al. (2008), who focused on the relation between net and gross N-mineralization.

Field survey

In each of the six beech forests, four to five plots were randomly selected in the forest interior. In each plot, samples were collected for laboratory incubation experiments in spring ($n=5$) and autumn ($n=4$). The organic layer was sampled in 25×25 cm squares. Branches and beech nut shells were left out because they were considered less important for N-cycling than leaves. In spring, fresh litter

Table 1 Site characteristics of Luxembourg beech forests on different soil

	Tx	pH	S1	S2	S3	L1	L2	L3
Organic layer								
pH _{KCl}	*	*	4.6	5.4	5.9	4.8	5.5	6.4
Mass (kg m ⁻²)	*	*	3.35	1.47	0.96	1.78	0.84	0.44
Thickness (cm)	–	–	4	2.5	2	4	2	2
Olson decomposition constant	*	*	0.17	0.33	0.61	0.31	0.60	1.28
C:N ratio	*	ns	21.8	20.8	21.4	24.6	25.7	28.3
Mineral topsoil								
pH _{KCl}	*	*	3.7	4.3	5.8	4.0	4.7	6.7
Sand content (%)	–	–	82	70	76	47	14	5
Carbon content (%)	*	*	5.0	2.1	4.1	3.7	5.1	8.4
C:N ratio	*	*	19.0	13.8	14.3	19.3	15.9	17.1
Bacteria (10 ⁶ CFU ml ⁻¹)	*	*	1.2	3.0	3.8	1.4	4.2	14.4
Actinomycetes (10 ⁶ CFU ml ⁻¹)	*	*	1.2	2.0	3.2	1.2	2.8	8.8

S1, S2, and S3 are sandy soils, and L1, L2, and L3 are loamy soils with low, intermediate, and high pH, respectively. Significant effects of texture (Tx; sandy or loamy) or pH (low, intermediate or high) in analysis of variance are given with an asterisk ($p<0.05$) ns not significant, – not tested because there were no replicate values

was separated from older organic matter, but left out of further analysis. The mineral topsoil was sampled within the 25×25 cm square, in three 90 cm³ metal rings with a depth of 5 cm. This depth may be a bit limited, especially in high pH soils, but it comprised the Ah completely, and response patterns did not differ when the upper 10 cm were used instead (Kooijman and Martinez-Hernandez 2009). Samples were stored at 4°C in the dark until analysis. In December, preceding and following the spring and autumn incubation experiments, litter fall and older ectorganic matter were collected in 25×25 cm squares, right after leaf fall ($n=5$). In the following spring, fresh samples of organic layer and mineral topsoil were collected for bacteria and actinomycetes counts ($n=5$).

Micromorphology

In spring, when samples were collected for incubation experiments, undisturbed samples of the upper soil were taken in each forest for micromorphological analysis. Samples were vertically put in Kubierna boxes to prepare thin sections (7 cm×5 cm×25 μm) for microscopical analysis of humus forms, soil fauna, and microorganisms. Analyses were conducted with an Olympus polarization microscope. To distinguish bacteria, which are rarely more than a few micrometers in length, high magnification (×1,000) and reflected light, rather than transmitted, were used. Based on earlier research on humus forms (Dijkstra and van Mourik 1996; van Mourik 2003) and the Guideline for Analysis and Descriptions of Soil and Regolith Thin Sections (Stoops 2003), we selected 22 soil micromorphological characteristics related to soil organic matter, ped characteristics, soil fauna, and microorganisms (Table 3). The 22 variables were semiquantitatively expressed on a scale from 0 to 3 (0 = not detected; 1 = low amounts or activity; 2 = moderate amounts or activity; 3 = high amounts or activity) and further analyzed with multivariate techniques.

Laboratory analysis

Fresh weight and gravimetric moisture content of organic layer and mineral topsoil were determined, and dry weight and bulk density calculated. Litter decay constants were calculated according to Olson (1963), as the ratio between dry weight of fresh litter and older ectorganic matter. After homogenization by hand, pH–KCl values were determined, using a 1:2.5 weight:volume ratio for mineral samples and 1:10 weight:volume ratio for organic samples. After drying (48 h at 70°C for organic and 105°C for mineral samples) and grinding of the subsamples, C and N contents were determined with a CNS analyzer (Westerman 1990).

Potential net N-mineralization in the organic layer and mineral topsoil was measured in a 6-week (spring) or 4-week

(autumn) laboratory incubation experiment. Fresh, homogenized samples were put into large petri dishes and stored at optimal gravimetric moisture levels (300% for organic and 50% for mineral soil samples; Tietema 1992) at 20°C in the dark. The petri dishes were stored in slightly open polyethylene bags with moist paper; moisture content was checked and corrected when necessary. Ammonium and nitrate of fresh and incubated samples were extracted with 50 ml 0.5 M K₂SO₄ solution, using the equivalent of 1.5 and 4.5 g dry material for organic and mineral samples, respectively, and measured on a continuous-flow analyzer (Westerman 1990). Net N-mineralization was calculated from differences in total inorganic N between incubated and fresh samples.

Microbial C and N were analyzed with chloroform fumigation and K₂SO₄ extraction (based on Brooks et al. 1985). Fumigated samples were flushed for 24 h with chloroform and extracted with 0.5 M K₂SO₄ immediately afterwards, to prevent microbial regrowth. In addition to ammonium and nitrate, DON and DOC were measured in fumigated and nonfumigated samples using a continuous-flow analyzer. Microbial C and N concentrations were calculated as differences between fumigated and non-fumigated samples. Microbial C and N in incubated samples generally did not differ from fresh ones, indicating that populations remained more or less stable and were left out from further analyses.

Bacteria and actinomycetes were measured in the organic layer and mineral topsoil, with standard plate count methods (ICMSF 2000). These methods only measure 1–10% of the microorganisms inhabiting the soil, but still give an overall view of differences between soil types. Duplicate samples of 10 g were extracted with sterile phosphate tamponed water in six steps of dilution and cultivated on sterile plates. For bacteria, plates with plate count agar were incubated for 48 h at 30°C. For actinomycetes, plates with actinomycetes agar were incubated for 6 days at 25°C. Values were expressed in 10⁶ CFU (colony forming unit) ml⁻¹.

Statistical analysis

Differences in site properties, microbial N, and net N-mineralization, which were measured in different seasons or years, were tested with three-way analysis of variance (ANOVA), with sampling period (repeated measurement), texture, and pH class as independent variables (Cody and Smith 1987); response variable patterns were generally not affected by sampling period. Microbial N and net N-mineralization showed more or less similar response patterns per kilogram soil per meter squared. To allow comparisons between organic layer and mineral topsoil, we chose for the latter and expressed values on an aerial basis. Differences in colonies of bacteria and actinomycetes were

tested with two-way ANOVA with texture and pH-class as independent variables because they were measured only once.

In order to classify soil micromorphological characteristics, the 22 parameters, expressed on a scale from 0 to 3, were used in a principal component analysis (PCA), within the program Canoco. As the number of sites was only six, and the scale semiquantitative, the approach should be seen as indicative only. After calculation of PC1 and PC2, correlations between micromorphology and site properties or factors related to N-dynamics were tested by using mean values of site properties and N-dynamics for each site as “environmental variables.” Negative and positive correlations of micromorphological characteristics, sites, site properties, and N-dynamics with PC1 and PC2 are given in Tables 4, 5, and 6, but for visibility reasons, a small overall selection is also presented in Fig. 3.

Results

Site factors and N-dynamics

As expected, pH of organic layer and mineral topsoil significantly differed between pH classes. However, in both layers, pH was affected by texture as well, with slightly higher values in loamy soil (Table 1). Similar to other site properties, differences in pH were not significant between sampling periods. Mass of the organic layer, litter decomposition constant, carbon content of the mineral topsoil, C:N ratio of the mineral topsoil, and soil bacteria and actinomycetes were all significantly affected by texture and pH, but C:N ratio of the organic layer only by texture. Mass of the organic layer and C:N ratio of the mineral topsoil were higher at low pH and in sandy soil. In contrast, litter decomposition constant and C:N ratio of the organic layer were highest in L3, the most calcareous loamy site. In this site, few litter remained in the organic layer, but what was left was relatively fresh and low-decomposed. In accord with high litter decay in the organic layer, C-content in the mineral topsoil was also higher at high pH and in loamy soil. In addition, bacteria and actinomycetes clearly increased from acid sandy to calcareous loamy soil as well.

Microbial N and net N-mineralization did not differ between spring and autumn, but were both significantly affected by pH and texture (Table 2; Fig. 1). In the organic layer, both microbial N and net N-mineralization were highest in acid, sandy soil (S1). In contrast, the mineral topsoil, and the two layers combined, showed higher microbial N in the calcareous, loamy L3. However, net N-mineralization did not follow that pattern and showed significantly lower values in L3 than in all other sites. In the mineral topsoil, net N-mineralization was further

Table 2 Potential effects of texture (sandy or loamy), pH (low, intermediate or high) and season (spring or autumn) on microbial N and net N-mineralization in laboratory incubation experiments, over a gradient of Luxembourg beech forests on different soil

		Texture	pH	Season
Microbial N (g m ⁻²)	Organic layer	**	*	ns
	Mineral topsoil	***	***	ns
	Both combined	***	**	ns
Net N-mineralization (g m ⁻² day ⁻¹)	Organic layer	***	***	ns
	Mineral topsoil	*	ns	ns
	Both combined	***	***	ns

s not significant ($p > 0.05$)

* p Value between 0.05 and 0.01; ** p value between 0.01 and 0.001;

*** p value between 0.001 and 0.0001

affected by texture, and showed generally higher values for sandy soil. High net N-mineralization in the mineral topsoil of L3, where C-content and microbial N were also higher, were thus expected, but did not occur. In fact, efficiency of N-mineralization, as indicated by net N-mineralization per unit microbial N, showed a clear decrease from S1 toward L3, in both organic layer and mineral topsoil.

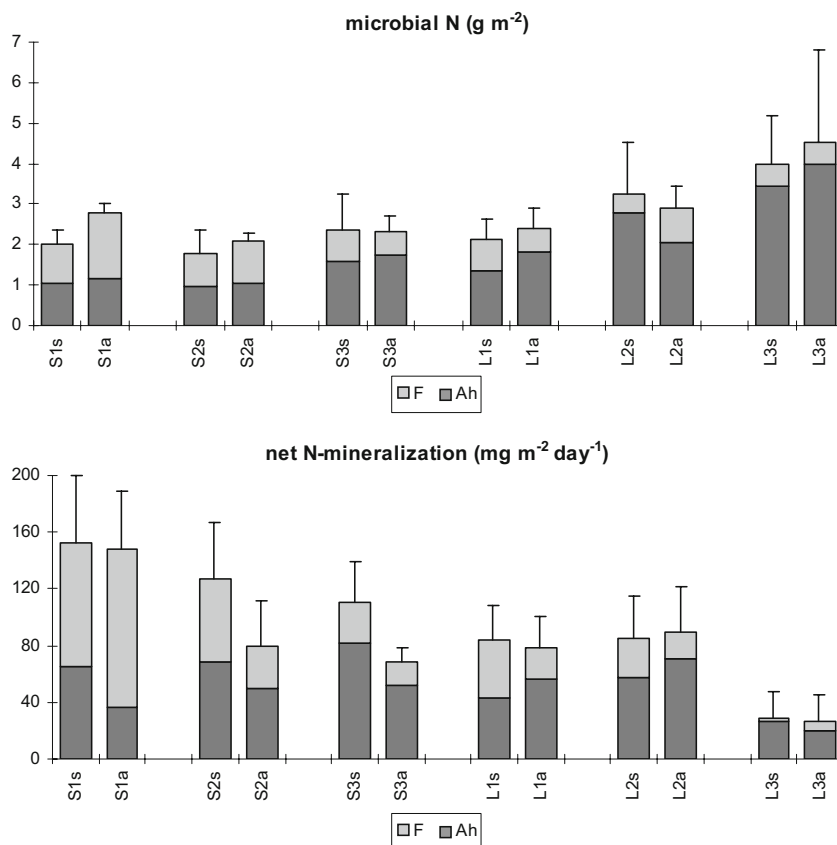
Micromorphology

The first step in micromorphology concerned the characterization of humus forms, organic matter and peds in the thin sections. In all sites, organic layers had a humiskel as soil skeleton, consisting of coarse particles of altered organic tissue and coarse organic aggregates. All mineral topsoils had a lithoskel, consisting of mineral particles. However, thickness of the organic layer and humus form differed between sites (Table 1). Thickness of the organic layer decreased from 4 cm in the acid S1 and L1 to 2 cm in the base-rich S3 and L3. Also, the organic layer consisted mainly of F-material in S1 and L1, but of relatively fresh litter in S3 and L3. Humus forms changed from mormoder in S1 and mullmoder in L1 to vermimull in all other sites.

Fine organic matter could be located in the soil in different ways: diffuse as intertextic humus, as organic cutans, in organic or mixed aggregates, or in fecal pellets from microarthropods and earthworms (Table 3). In the litter layer, humus was generally only found as fecal pellets. Humic fecal pellets in litter were especially abundant in the soils with high litter decay S3, L2, and L3. In the F-horizon, humic material can also be present in complex peds. Like the litter layer, humic fecal pellets in the F-horizon were most abundant at high pH. However, complex peds were mainly found on sandy soil, where the organic layer is more extensive than on loamy soil.

In the mineral topsoil, organic material was located as intertextic humus, organic cutans, fecal pellets, and peds.

Fig. 1 Microbial N and potential net N-mineralization in laboratory incubation experiments in spring and autumn in Luxembourg beech forests on different soil. S1, S2, and S3 are sandy soils, and L1, L2, and L3 are loamy soils with low, intermediate and high pH, respectively; *s* spring, *a* autumn, *F* organic layer, *Ah* mineral topsoil (0–5 cm). Mean values ($n=4-5$) are given for organic layer and mineral topsoil separately; SDs are those of the two layers combined



Fecal pellets were detected within old root structures, but also within peds in different stages of development. Intertextic material occurred in all sites, but organic cutans only in acid and/or sandy soil. Like in litter and F-horizon, fecal pellets in the mineral topsoil were especially abundant at high pH and/or loamy soil. Ped characteristics in the mineral topsoil further illustrated differences between soil types. Peds are constructed by soil fauna and microorganisms by compilation of organic matter. In acid soil, the organic ped substances were small, highly decomposed masses of which the original structure was hardly recognizable, and probably derived from fused fecal pellets. Also, acid soil peds contained a lot of hyphae, which indicates that organic matter was already attacked by (primary) fungi before soil fauna started consumption and fragmentation. At high pH, peds contained more recognizable and less-decomposed organic matter, probably because earthworms often transport consumed organic material to deeper parts of the soil. Bacteria further contributed to ped formation by the production of collating agents, which glue the different elements together.

Decomposition of fresh organic material is closely linked to soil organisms. Soil fauna, such as earthworms and microarthropods (springtails, mites, and enchytraeids), and microorganisms, such as bacteria and fungi, both play a role. Macroscopic decomposers were not

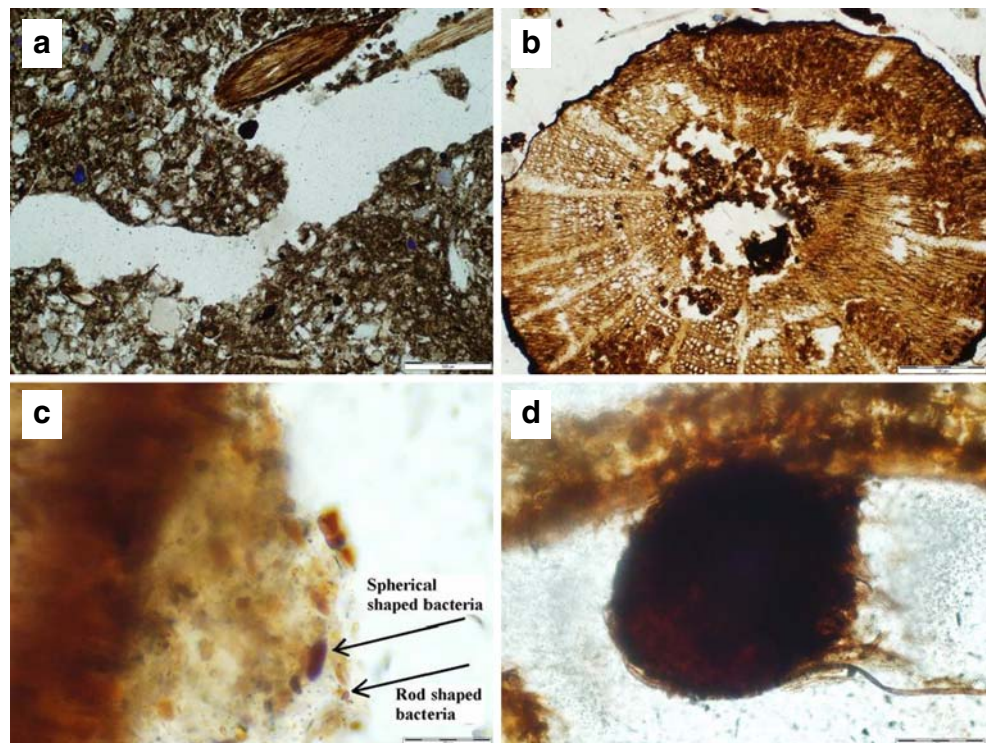
found in the thin sections, but their presence could be judged by traces of their activity, such as earthworm channels (Fig. 2a) and fecal pellets (Fig. 2b). Bacteria could be seen with high magnification and were present in rod-shaped and spherical form, often in colonies (Fig. 2c). Fungi were clearly visible in the thin sections, as primary fungi attacking leaf fragments, secondary fungi attacking fecal pallets (Fig. 2d) or soil peds, and ectomycorrhiza around plant roots. Earthworm activity was highest at high pH and in loamy soil. Earthworms were thus absent from the acid, sandy S1, but abundant in S3, L2, and L3. Microarthropods were also more active in loamy soil with high pH, especially in litter layer and mineral topsoil. In the F-horizon, however, microarthropod activity showed a peak under acid conditions, probably because the organic layer was more extensive here. Bacteria showed a clear preference for high pH and loamy soil. Bacterial activity was high in L2 and L3, but not at all observed in S1 and L1. In contrast, fungi were more abundant in acid soil. Primary fungi in litter and F-horizon, and secondary fungi in F-horizon and mineral topsoil all showed a peak in S1 and L1. Mycorrhiza, however, did not respond to the soil gradient. Instead, ectomycorrhiza were present in sites with sparse undergrowth, but absent from sites with high cover of the grass *Melica uniflora*, independent of pH and texture.

Table 3 Micromorphological analysis of six Luxembourg beech forests on different soil

	S1	S2	S3	L1	L2	L3
Soil organic matter and ped characteristics						
Humic fecal pellets in litter layer	1	1	3	1	3	3
Humic fecal pellets in F-layer	2	1	3	2	3	3
Complex peds in F-layer	2	2	2	1	1	0
Intertextic humic material	2	2	2	2	2	2
Organic cutans in mineral topsoil	1	0	1	1	0	0
Humic fecal pellets in mineral topsoil	2	1	3	2	3	3
Amount of peds in mineral topsoil	1	2	2	2	3	3
Strength of peds in mineral topsoil	3	2	2	1	3	3
Recognizable organic material in peds	1	2	3	2	3	3
Fungal hyphae in peds mineral topsoil	3	3	1	3	1	0
Fecal pellets in peds mineral topsoil	2	2	2	2	0	0
Soil fauna and microorganisms						
Earthworm channels	1	1	3	2	3	3
Earthworm chambers	0	1	3	2	0	3
Microarthropod activity in litter layer	1	1	1	1	3	3
Microarthropod activity in F-layer	3	2	2	3	2	2
Microarthropod activity in mineral topsoil	1	1	3	1	3	3
Bacteria	0	1	1	0	3	3
Primary fungi in litter layer	3	3	1	3	2	1
Primary fungi in F-layer	3	2	1	3	2	1
Secondary fungi in F-layer	3	3	1	3	1	0
Secondary fungi in mineral topsoil	3	1	0	3	1	0
Mycorrhiza	1	0	0	1	1	1

S1, S2, and S3 are sandy soils, and L1, L2, and L3 are loamy soils with low, intermediate, and high pH, respectively. All parameters are expressed on a 0–3 scale: 0 not detected, 1 low activity or amount, 2 moderate activity or amount, 3 high activity or amount. This semiquantitative approach is used as a basis for further (multivariate) analysis of thin section characteristics

Fig. 2 Selection of micromorphological characteristics in thin sections. **a** Channel of an earthworm, F-Ah layer site L2 (scale bar indicates 500 μ m). **b** Fecal pellets within a root structure, F layer site S1 (scale bar indicates 500 μ m). **c** Bacterial activity, Ah layer site L3 (scale bar indicates 20 μ m). **d** Secondary fungi attack on a fecal pellet, F layer site L1 (scale bar indicates 50 μ m)



Principal component analysis

Micromorphological characteristics were analyzed with principal component analysis. The first PCA-axis (PC1) had relatively high eigenvalue (0.70), which suggests that micromorphological characteristics (and sites) clearly differed across the soil gradient (Table 4; Fig. 3). The second axis (PC2), which had an eigenvalue of only 0.15, was much less important. Primary and secondary fungi in L, F, and Ah were grouped together with fungal hyphae in peds on the negative part of PC1. Microarthropod activity in the F-horizon belonged to this group as well. The other, positive end of PC1 was occupied by earthworm activity, microarthropod activity in litter layer, and mineral topsoil, fecal pellets in all soil layers, and bacteria. The first axis thus clearly separated variables related to fungi from those of earthworms, bacteria,

Table 4 Principal component analysis (PCA) of thin section characteristics and sites in six Luxembourg beech forests on different soil

	PC1	PC2
Micromorphological characteristics		
Secondary fungi in F-layer	-0.99	-0.02
Fungal hyphae in peds mineral topsoil	-0.99	-0.02
Primary fungi in litter layer	-0.92	-0.33
Primary fungi in F-layer	-0.84	-0.39
Secondary fungi in mineral topsoil	-0.84	-0.29
Fecal pellets in peds mineral topsoil	-0.80	0.57
Microarthropod activity in F-layer	-0.74	-0.09
Complex peds in F-layer	-0.61	0.29
Organic cutans in mineral topsoil	-0.49	0.49
Intertextic humic material	0.00	0.00
Mycorrhiza	0.06	-0.67
Strength of peds in mineral topsoil	0.45	-0.60
Earthworm chambers	0.46	0.78
Microarthropod activity in litter layer	0.80	-0.57
Amount of peds in mineral topsoil	0.83	-0.23
Humic fecal pellets in mineral topsoil	0.83	-0.01
Humic fecal pellets in F-layer	0.83	-0.01
Bacteria	0.89	-0.40
Earthworm channels	0.90	0.12
Recognizable organic matter in peds	0.91	0.18
Microarthropod activity in mineral topsoil	0.97	0.04
Humic fecal pellets in litter layer	0.97	0.04
Sites		
S1	-0.60	-0.34
L1	-0.48	0.20
S2	-0.41	0.07
S3	0.33	0.88
L2	0.43	-0.78
L3	0.73	-0.04

Values are negative or positive correlations with the first principal component (PC1; eigenvalue 0.70) and with the second (PC2; eigenvalue 0.15). S1, S2, and S3 are sandy soils, and L1, L2, and L3 are loamy soils with low, intermediate, and high pH, respectively

and microarthropod activity in general. The sites were grouped accordingly. S1, L1, and S2 were found on the fungi-part of PC1, and S3, L2, and L3 on the earthworm–bacteria part. The sites were further separated along PC2, but as the eigenvalue was so low, this is of limited importance.

Site properties were also clearly separated along PC1 (Table 5; Fig. 3). Mass and thickness of the organic layer correlated with the negative (fungi) end, and so did the fraction of sand. In contrast, pH of organic layer and mineral topsoil, litter decomposition constant, C-content of the mineral topsoil, and colonies of bacteria and actinomycetes clearly correlated with the positive (earthworm–bacteria) end of PC1.

Factors related to N-dynamics also clearly correlated with PC1 (Table 6; Fig. 3). In the organic layer, both microbial N and net N-mineralization pointed in the direction of the fungi end of the axis. In the mineral topsoil, microbial N clearly correlated with the earthworm–bacteria end of PC1. However, net N-mineralization did not show preference for earthworms and bacteria at all. In fact, net N-mineralization in the mineral topsoil even slightly pointed toward fungi. Also, efficiency of N-mineralization per unit microbe clearly correlated with the fungi end of PC1 instead of bacteria in both organic layer and mineral topsoil.

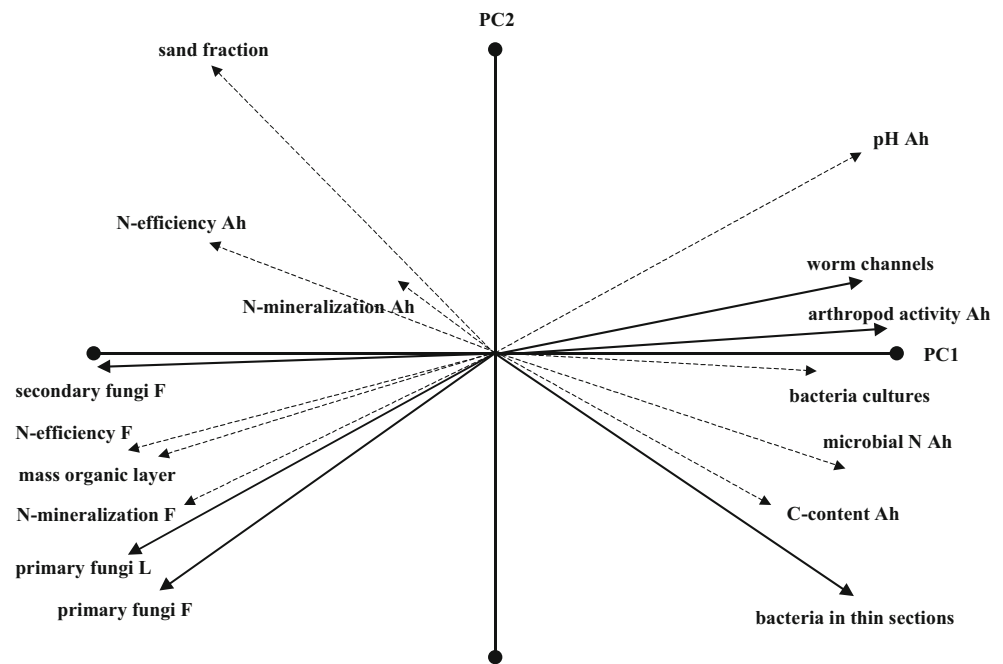
Discussion

Micromorphological analysis

The objective of this paper was to further unravel relationships between net N-mineralization and soil organisms, by combining characteristics of microbial behavior with micromorphology over a soil gradient in pH and texture. The approach was only semiquantitative, but the clear separation of thin section characteristics into two groups supports the idea that there are two main pathways in litter breakdown (e.g., Green et al. 1993; Aerts and Chapin 2000; Ponge 2003): one related to activity of earthworms, microarthropods, and bacteria, and the other to primary and secondary fungi in L, F, and Ah, and hyphae in fecal pellets. The fungal and earthworm–bacteria pathways can coexist to some extent (Moore et al. 2005), but separately lead to clearly different mull or mormoder humus forms.

In the earthworm–bacteria pathway, soil animals fragment and consume most of the litter, which leads to rapid decay and mull humus forms. Organic matter is mainly stored in the mineral soil, which is supported by the high C-content, especially in the most calcareous soil. Earthworms consume a lot of litter, especially anecic species such as the common *Lumbricus terrestris* L., and produce stable microaggregates enriched in both organic matter and clay (e.g., Marhan and Scheu 2005; Pulleman et al. 2005). Organic matter content

Fig. 3 PCA diagram (based on micromorphology) with a selection of thin section characteristics (*solid lines*) and response factors related to site conditions and N-dynamics (*dashed lines*). Eigenvalues of PC1 and PC2 are 0.70 and 0.15, respectively. N-efficiency = net N-mineralization per unit microbial N



may be further enhanced by stabilization of organic matter by calcium carbonate (Duchaufour 1982; Grünewald et al. 2006). In the earthworm–bacteria pathway, microarthropods were abundant as well, in both litter layer and mineral topsoil. Microarthropod communities (springtails, mites, and enchytraeids) consist of many different species, with diverse food sources such as roots, detritus, fungi, nematodes, etc. (Coleman and Crossley 1996; Moore et al. 2005). However, in thin sections, it may be possible to estimate overall activity, but microarthropod diversity is difficult to judge because most fecal pellets are undifferentiated through microbial attack and may fuse together (Davidson et al. 2004). Bacteria were abundant in the earthworm–bacteria

pathway as well. Bacteria are the most numerous inhabitants of the soil and are capable of very rapid reproduction by binary fission. They generally consume relatively high degradable food, such as simple sugars and proteins, and can be found around roots, fecal pellets, and mucus secretions in the burrows of earthworms (Coleman and Crossley 1996). Bacteriophagous nematodes and protozoa were probably important as well (Moore et al. 2005), but could not be traced in the thin sections.

In the fungal pathway, earthworm activity is restricted, which leads to accumulation of litter in ectorganic horizons and mormoder humus forms (Ponge 2003). Litter decay seems mainly due to primary fungi, which can move through the organic layers via growing hyphae and attack numerous substrates (Coleman and Crossley 1996). Microarthropods are mainly active in the F-horizon, where fungi are abundant, substrate quality (N-content) has improved compared to fresh litter, and plant roots are found as well. Although microarthropods in turn produce fecal pallets which can be used as food source for secondary fungi, it is not clear to which extent they are crucial to (further) litter breakdown. Humus form development under Scots pine was similar when microarthropods were excluded due to toxic heavy metals (Dijkstra 1998). In the mineral topsoil, microarthropods were present, if not as much as in the earthworm–bacteria pathway. However, secondary fungi, feeding on fecal pellets and soil aggregates were most abundant.

Table 5 Relationship between micromorphology and site factors of six Luxembourg beech forests on different soil

	PC1	PC2
Mass organic layer (kg m^{-2})	-0.85	-0.17
Thickness organic layer (cm)	-0.85	-0.09
Sand content (%)	-0.71	0.47
C:N ratio organic layer	-0.35	-0.31
C:N ratio mineral topsoil	0.65	-0.39
Carbon content mineral topsoil (%)	0.68	-0.25
Bacteria mineral topsoil (10^6 CFU ml^{-1})	0.79	-0.03
Actinomycetes mineral topsoil (10^6 CFU ml^{-1})	0.81	0.03
Olson litter decomposition constant	0.90	0.05
pH organic layer	0.90	0.25
pH mineral topsoil	0.90	0.33

Values are negative or positive correlations with PC1 and PC2 (eigenvalue 0.70 and 0.15, respectively), as calculated with the 22 thin section characteristics (Table 4)

Soil organisms and site conditions

The clear association of the earthworm–bacteria and fungal pathways with site factors was also in accord with

Table 6 Relationship between micromorphology and N-dynamics of six Luxembourg beech forests on different soil

	PC1	PC2
Efficiency of net N-mineralization in organic layer	-0.92	-0.16
Net N-mineralization organic layer ($\text{mg m}^{-2} \text{day}^{-1}$)	-0.78	-0.25
Microbial N in organic layer (g m^{-2})	-0.74	-0.22
Efficiency of net N-mineralization in mineral topsoil	-0.72	0.18
Microbial C:N ratio in mineral topsoil	-0.62	-0.42
Microbial C:N ratio in organic layer	-0.53	-0.68
Nitrification organic layer (%)	-0.40	0.24
Net N-mineralization mineral topsoil ($\text{mg m}^{-2} \text{day}^{-1}$)	-0.25	0.12
Nitrification mineral topsoil (%)	0.34	0.68
Microbial N in mineral topsoil (g m^{-2})	0.86	-0.19

Values are negative or positive correlations with PC1 and PC2 (eigenvalue 0.70 and 0.15, respectively), as calculated with the 22 thin section characteristics (Table 4). Efficiency of net N-mineralization is expressed as $\text{mg g}^{-1} \text{microbial N day}^{-1}$

literature. Earthworms are indeed generally rare in acid soil (e.g., Pop 1997; Davidson et al. 2004), which may be related to a lack of base cations. Calciferous glands in earthworm guts supposedly could neutralize acidic soil, but recent studies suggest that precipitation of calcium carbonate is mainly used to reduce high tissue CO_2 concentrations and high calcium in ingested soil (Iglesias Briones et al. 2008). Earthworms generally prefer loamy soil because the abrasive action of sand grains damages their skin, but they also need high soil moisture to prevent desiccation. In the highly diverse group of microarthropods, there are both acidophilic and calciophilic species. However, even acidophilic species seem to have an optimum at high pH (Hågvar 1990). Microarthropods may also be affected by soil texture, as species associated with bacterial and fungal pathways are generally spatially separated (Moore et al. 2005), but they could respond to food source as well.

Although acidophilous bacteria exist, many soil species prefer more base-rich conditions (Blagodatskaya and Anderson 1998; Bååth and Anderson 2003). Actinomycetes, a major group of soil bacteria, even prefer alkaline soil with pH between 6.5 and 8. Fungi, on the other hand, are mainly found in acid soil. They can occur under base-rich conditions (Ponge 2003), but are possibly outcompeted by the more abundant bacteria and partly preferentially consumed by earthworms (Tiwari and Mishra 1993; Bonkowski et al. 2000). Bacteria and fungi also clearly differ in preference for soil texture. Bacteria live in smaller, water-filled pores (Moore et al. 2005) and are more abundant in loamy soil (Hassink et al. 1993; Hassink 1994). Fungi, on the other hand, are strictly aerobic and live in larger, air-filled pores, which are more common in sandy soil (Moore et al. 2005).

Soil organisms and N-dynamics

The earthworm–bacteria and fungal pathways were also associated with N-dynamics, albeit in a different way than generally assumed (Aerts and Chapin 2000; Ponge 2003; Seeber et al. 2008). In our laboratory study, fungi-dominated soil with low litter decay showed high instead of low net N-mineralization. In contrast, bacteria-dominated soil with high litter decay showed low instead of high net N-mineralization in both organic layer and mineral topsoil. N-cycling is driven by the depolymerization of N-rich polymers by microbes, and in low-N systems, plants may even use organic N as N-source (Schimel and Bennett 2004). Nevertheless, mineralization of N to inorganic components is still considered important and supposed to increase when biological activity is high. Yet, like in our study, several field and laboratory studies have reported higher instead of lower net N-mineralization in acid than calcareous soils (Zöttl 1960; Davy and Taylor 1974; Verhoeven et al. 1988, 1990; Kooijman and Besse 2002). Also, agricultural loamy soils, with supposedly higher biological activity, showed lower net N-mineralization than sandy soils (Hassink et al. 1993; Hassink 1994). In the above experiments, earthworms were usually excluded. Earthworms are known to increase net N-mineralization (e.g., Wolters and Stickan 1991; Scheu 1997; Marhan and Scheu 2005; Postma-Blaauw et al. 2006) and may even increase plant productivity (Seeber et al. 2008). However, these results are usually based on presence and absence of earthworms under otherwise similar conditions and do not necessarily show that soils with high earthworm activity by nature should have higher net N-mineralization than natural low-earthworm soils. In Wolters and Stickan (1991), soil pH was more important than earthworms, and plant N-uptake was higher from acid than from lime-rich soil, independent of earthworms. Also, Marhan and Scheu (2005) found that leaching of mineralized N from wormcasts strongly increased in arable soil, but not in lime-rich forest soil. In addition, in our study, the most calcareous L3 had consistently lower N-content in fresh litter over the past 4 years than the acid sandy S1 ($0.89 \pm 0.08\%$ vs $1.15 \pm 0.07\%$). This suggests that, even if earthworms increase N-availability to the vegetation in soils with high litter decay, this may not always be sufficient to compensate the potentially low net N-mineralization.

Differences in N-cycling between soils with high and low litter decay may be due to the earthworm–bacteria and fungal pathways indirectly, via distribution of organic matter over the soil profile, which differs between mull and mormoder humus forms (Ponge 2003). However, net N-mineralization may also be affected directly via nutritional requirements of the soil organisms involved. In the organic layer, the decrease in net N-mineralization from

fungi to earthworm- and bacteria-dominated soil is clearly related to declining mass of the organic layer due to higher litter consumption by soil fauna. However, part of the decline is also attributed to lower efficiency of N-mineralization per unit microbe. In the organic layer of earthworm- and bacteria-dominated soil, low efficiency of N-mineralization may be due to the relatively fresh litter with low N-content, which may induce N-immobilization rather than net release (Swift et al. 1979). In contrast, the organic layer of fungi-dominated soil may show high net N-mineralization per unit microbe because substrate quality of the more-decomposed organic matter is higher (higher N-content). However, high efficiency of N-mineralization may also be due to low N-requirements of fungi. Fungi generally have higher C:N ratios than bacteria. Microbial C:N ratio is usually estimated around four for bacteria, and ten for fungi (Moore et al. 2005), but may even amount to 20 for the latter (Wallander et al. 2003). Fungi may have lower N-requirements than bacteria because of their structure and slower life cycle, but also because they use carbohydrates as osmoregulators rather than amino acids (e.g., Measures 1975; Kuehn et al. 1998).

Low N-requirements by fungi may also explain the relatively high net N-mineralization found in the mineral topsoil of more acid sites. In contrast, low net N-mineralization in the most calcareous soil may be due to high bacterial N-demand. In bacteria-rich soils, net N-mineralization may have been underestimated to some extent because earthworms were excluded and deeper soil layers not taken into account. However, modeled gross N-mineralization suggested that calcareous, bacteria-rich soil had high biological activity even if earthworms were absent (Kooijman et al. 2008). Unfortunately, microbial N-demand was also high, and immobilized 80% of the N net released, which explained why net N-mineralization was so low. A large part of bacterial N may be recycled, but after some time probably accumulates in low-degradable substances and stable soil organic matter (Sjöberg and Persson 1998). This is supported by a long-term field experiment, where storage of N in the soil was higher in limerich than in acid grasslands (Phoenix et al. 2003).

Concluding remarks

The micromorphological analysis clearly suggests that retarded decomposition is associated with the fungal pathway and high litter decay with earthworms and bacteria. However, differences in net N-mineralization may be smaller than expected from such large differences in litter breakdown. In fungi-dominated soil, net N-mineralization may be relatively high because low biological activity and gross N-release are compensated for by low microbial N-requirements. In contrast, in soil dominated by earthworms and bacteria, biological activity may be high, and earthworms even have fertilizing effects, but high gross N-release may be counter-

acted by high microbial N-demand. Fungal and earthworm-bacteria pathways may thus fundamentally differ in decomposition and N-cycling, but both may provide equivalent strategies to sustain N-availability to the vegetation.

Acknowledgements We like to thank Leo Hoitinga, Greet Kooijman, Benito Martinez, Leen de Lange, Piet Wartenbergh en Joke Westerveld for assistance in the field and laboratory, and Jan Sevink, Annemieke Smit and three anonymous reviewers for encouraging discussions and comments.

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