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Shifts in physiological capabilities of the microbiota during the decomposition of leaf litter in a black alder (*Alnus glutinosa* (Gaertn.) L.) forest

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Abstract

Physiological capabilities of culturable bacterial and fungal communities were studied over 12 months during leaf litter decomposition in a black alder (*Alnus glutinosa* (Gaertn.) L.) forest at a eutric-wet and dystric-dry Histosol. Microbial biomass content, basal respiration rate, metabolic quotient, β-glucosidase and protease activity and abiotic properties of the litter were also considered as 'integral' microbiological characteristics since both bacterial and fungal physiological capabilities were included. The number of copiotrophic and proteolytic bacteria were positively correlated while the numbers of cellulolytic and lipolytic bacteria were negatively correlated. Fungal enzymatic potentials were generally positively linked with each other and with the corresponding physiological capabilities of bacteria. Cellulolytic bacterial numbers were positively associated with fungal polygalacturonase and lignolytic activity. In contrast, numbers of lipolytic bacteria and the lipolytic fungal potential were negatively correlated. The fungal communities appeared to play a predominant role in litter breakdown at the early stages whereas bacteria completed the mineralisation. Contrary to the integral microbiological characteristics, data on physiological capabilities of bacteria and fungi connected with abiotic properties were poorly correlated. The connectance between bacterial and fungal capabilities, integral microbiological characteristics and abiotic factors varied between 0.06 and 0.51 and correlations were generally lower at the eutric-wet site. At the dystric-dry site, mineralisation rates were water-limited during the summer months, and protease and β-glucosidase activity related to the microbial biomass were also reduced to less than 20% of the maximal values.

Keywords: Bacteria; Fungi; Interaction; Litter; Microbial Metabolism; Succession

1. Introduction

Microorganisms are the dominant biotic structural components in soil and have high biomass-specific activities. Thus, these organisms play a predominant role in the mineralisation of organic compounds. This is particularly evident during leaf litter decomposition, which is of crucial importance for elemental cycling in natural ecosystems. During litter decay, a succession of organisms occurs which is represented by the adjusting composition and interaction of biological communities involved in biodegradation (Frankland, 1998). This suggests that different catabolic capabilities are sequentially required to complete the decomposition process.

Most studies on succession have been confined to single biological groups such as fungi, bacteria or protozoa (Swift, 1982). Such studies showed that highly active, zymogenous and *r*-selected organisms dominate the beginning of decomposition whereas autochthonous or *K*-selected, more energy-efficient organisms occur during later stages (Zvyagintsev, 1994). Simultaneously, a decrease in biomass content, respiration rate and β-glucosidase activity rate (representing 'integral' microbiological characteristics since biomass components and activity of single biological groups are included) was observed during the course of decomposition (Dilly and Munch, 1996, 1998). This suggests a decline in litter quality and transformation intensity. Apart from analysing either integral microbiological

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characteristics (Dilly and Munch, 1996) or single microbial groups (Kjøller and Struwe, 1980; Kjøller et al., 1985), remarkably little is known about the interrelations between bacteria, fungi and integral components (e.g., Kjøller et al., 1985); reasons being that their determination is laborious, requires expertise from different fields and, furthermore, changes in the respective dynamics of the organism modules do not usually appear well connected to material and energy flows (Pickett et al., 1994). Coupled investigations may, however, provide the required data and reveal fundamental ecological information about the nutritional physiology of the microbiota and their eco-physiological adjustment to environmental conditions. Such investigations may elucidate metabiotic interactions between microorganisms and functional groups of organisms in plant litter systems. Metabiosis is 'a form of ecological dependence in which one organism must modify the environment before the second is able to live in it' (Waid, 1997).

The interdisciplinary research program 'Ecosystem Research in the Bornhöved Lake District', which began in 1988 and ended 1999, was aimed at analysing and modelling structures, dynamics and functions of terrestrial and aquatic ecosystems. The aim of this investigation was to evaluate temporal coincidences of the physiological capabilities of bacterial and fungal communities together with integral microbiological characteristics and abiotic controlling factors during the course of decomposition of leaf litter in a black alder (*Alnus glutinosa* (Gaertn.) L.) forest during the breakdown of organic matter. The experiment thus encompasses the population and ecosystem paradigms (Pickett et al., 1994) and focussed biomass components and activities of the microbial capabilities. The data from the 12 month experiment facilitates the investigation of the interdependence of biotic components and the role of abiotic controlling factors. The results will be discussed in context with published data from the same experimental plots with reference to changes in the degradative potential of fungi (Rosenbrock et al., 1995) and microbial C content, basal respiration rate, β-glucosidase and casein-hydrolysing activity rates and abiotic properties (Dilly and Munch, 1996).

2. Materials and methods

2.1. Sites and soils

The experimental black alder forest was located approximately 30 km south of Kiel in northern Germany (54° 06′N, 10° 14′ E). The geological basis was formed during the Pleistocene glaciation ('Ostholsteinisches Hügelland'). In 1992, mean annual rainfall at the local meteorological station was 756 mm and the average annual air temperature 9.2°C with a minimum monthly temperature of −1.6°C in January and a maximum of 18.4°C in June and July. Temperature and air humidity (determined with Hyromer® humidity sensor; rotronic, Bassersdorf, Switzerland) were also considered for correlation to the microbiological data since experiments with the leaves in continuous flow systems showed sensitively increasing respiration rates when humidifying the air (data not shown).

Black alder (*Alnus glutinosa* (L.) Gaertn.) was the dominant plant in the canopy. Beech (*Fagus sylvatica* L.), oak (*Quercus robur* L.) and some shrubs, mainly hazel (*Corylus avellana* L.), in the understorey contributed up to 20% of the examined litter. Two different sites of the alder forest were selected: A eutric-'wet' site with a thin canopy, connected by a reed belt (*Phragmites australis* Trin. *ex* Steud.) with the eutrophic lake 'Belauer See', and a dystric-'dry' site approximately 50 m inside the forest and at the foot of a hill. The soils (0–20 cm depth) of these sites had pH values measured in de-ionised water of approximately 6.0 and 3.5, and were classified as eutri-terric and dysti-fibric Histosols respectively (FAO, 1988; Soil Survey Staff, 1988).

2.2. Experimental procedure

During the litter-fall period in 1991, leaf litter (approximately 80% alder leaves) was collected on nylon nets throughout the experimental alder forest. The litter was slowly dried at 30°C in the laboratory and mixed. Approximately 10 g dry litter was placed in 10 × 20 cm nylon bags with 5 mm mesh size. At the end of December the bags were distributed on the *L* horizon at the two sites of the forest and harvested every 28 days. Four replicates were sampled at the beginning of the experiment. As litter degradation proceeded, the number of harvested bags increased up to 12 to obtain sufficient material for all analyses. The content of the bags was first weighed and afterwards randomly distributed enabling each partner to analyse three to five independent replicates. The experiment studying both single capabilities and integral characteristics ran for 12 samplings. Samples were stored at 4°C until analysed.

2.3. Bacteria and fungi

The physiological capabilities of nine bacterial groups were quantified on solid and liquid media. The litter was cut into squares of approximately 5 × 5 cm and shaken in 0.2% (w/v) sodium hexametaphosphate (pH 7.0). An adequate dilution of the litter suspension was transferred to the following media: (1) broth containing 0.5% (w/v) peptone of meat and 0.3% meat extract (pH 8.0) to estimate the total number of culturable, copiotrophic (adjusted to high substrate concentrations) bacteria, (2) agar with 1% (w/v) starch for amylolytic bacteria (Wollum, 1982), (3) liquid medium with 0.5% (w/v) xylan for xylanolytic bacteria (Näveke and Tepper, 1982), (4) liquid protein medium containing 12% (w/w) gelatine with 0.5% (w/v) meat peptone and 0.3% meat extract (pH 7.0) for proteolytic bacteria, (5) agar plate containing 1% (v/v) Tween 20, 1% (w/v) Difco-Bacto-Pepton, 0.5% sodium chloride, 0.01% $CaCl_2$ (pH 6.0) for lipolytic bacteria, (6) liquid media with

filter paper for cellulolytic bacteria (Omelianski, 1902), (7) agar plate containing sodium polypectate for pectolytic bacteria (Bieleit, 1990), (8) liquid media containing ammonium sulphate and testing of nitrite development for ammonium-oxidising bacteria, and (9) liquid media containing sodium nitrite and testing of nitrate development for nitrite-oxidising bacteria. Fungal growth was suppressed by adding 150 mg cycloheximide per 1000 ml media.

To estimate the fungal capabilities considering mainly active fungal components, litter was cut into pieces of 5×5 mm and washed to remove the inactive spores. To suppress bacterial growth, the litter squares were washed with 150 ml aqueous solution of chloramphenicol (50 mg l^{-1}). For each site, 40 washed litter pieces were placed on malt extract agar (MEA) and agar medium prepared with cold soil extract (CSEA). Strains isolated more than once were tested with regard to the following NINE enzymatic potentials: (1) amylase (Kreisel and Schauer, 1987), (2) protease (Hankin and Anagnostakis, 1975), (3) lipase (Hankin and Anagnostakis, 1975), (4) xylanase (Schinner and von Mersi, 1990; mod.), (5) polygalacturonase, (6) pectinase (Durrands and Cooper, 1988; mod.), (7) cellulase (Smith, 1977) and lignin-oxidation (distinguishing (8) 'non-specific' phenol-oxidising potential after Bavendamm (1928) and (9) 'specific' laccase potential after Niku-Paavola et al., 1990). More details on the methodology were described by Rosenbrock et al., 1995. Altogether, 5–17 strains were tested at each sampling date. Low numbers of strains occurred during summer.

2.4. Integral microbiological characteristics and abiotic data

Integral microbiological characteristics were analysed as follows: The microbial biomass content was estimated by determining the substrate-induced respiration rate (Anderson and Domsch, 1978), the basal respiration rate at 22°C and non-restricted water supply, β-glucosidase activity rate with the substrate saligenin-β-D-glucopyranoside (Hoffmann and Dedeken, 1965; mod.) and protease activity rate using casein (Ladd and Butler, 1972; mod.). Per replicate, 1 g dry mass (with more than 2.5 g H_2O g^{-1} dry litter), 0.2 g fresh litter and 0.4 g fresh litter was used for the estimation of basal and substrate-induced respiration, β-glucosidase and protease activity respectively. Further information is available in Dilly and Munch (1996).

Dry matter was measured by drying at 105°C, C_{org} content by loss at ignition at 500°C and pH value in suspension of 1 g of fresh leaves in 10 ml of de-ionised water. Additional experimental information is available in Dilly and Munch (1996).

2.5. Statistics

Statistical analyses were performed using SigmaStat (Jandel Scientific, Erkrath, Germany). Simple and multiple linear regression analyses were considered to estimate parameters controlling bacterial and fungal capabilities and integral microbiological characteristics. Spearman rank correlations were used to evaluate the interrelationships between microbiological characteristics ($p < 0.05$) hypothesising that: (1) temporal coincidences of the physiological capabilities as shown by positive correlation coefficients suggest that the components presumably degrade the target compounds at the same time whereas, (2) negative correlations indicate varying importance for the degradation of the respective compounds during different stages of the decomposition.

The connectance (Moore and Hunt, 1988; Cohen et al., 1990) between physiological capabilities of bacterial and fungal communities, integral microbiological characteristics and abiotic factors was calculated by $2L\ (S, S')^{-1}$ with L = number of positive correlations and S, S' = number of two considered collectives. S' was replaced by (S-1) for estimating the connectance within the microbial capabilities. Values of 1.00 indicate that links exist between all considered components. Best subset regression was finally performed to determine the most important bacterial and fungal capabilities and microbial components and rejecting auto-correlation between microbiological components and target variables.

3. Results and discussion

3.1. Bacteria

The positive correlation coefficients between the copiotrophic bacteria and proteolytic bacteria at the two sites (Table 1) indicate the association between the two components. The media used for the estimation of copiotrophic and proteolytic bacterial numbers contained similar substrates and may have contributed to the high correlation coefficients. The xylanolytic bacteria were positively correlated to proteolytic and copiotrophic bacteria at the dry and wet site respectively. In contrast, cellulolytic bacteria negatively peaked with those degrading hydrophobic compounds proposing replacement in bacterial metabolism between lipoid and non-lipoid compounds. Proteolytic bacteria coincided with their product consumers, the ammonium-oxidisers, at the wet site where the decomposition proceeded more rapidly.

Table 1
Spearman rank order correlation coefficients between physiological capabilities of bacterial communities during decomposition of leaf litter at two sites in the alder forest of the Bornhöved Lake district ($n = 12$ [samplings], $p < 0.05$). Abbreviations: xylanolytic xyl. lipolytic lip. proteolytic pro. ammonium-oxidising amm. copiotrophic cop. cellulolytic cel

		Xyl	Lip	Pro	Amm
Dry site	Cop			0.71	
	Xyl			0.76	
	Cel		−0.61		
Wet site	Cop	0.62		0.80	
	Xyl				
	Cel		−0.69		
	Pro				0.68

3.2. Fungi

In contrast to poor connectance between the bacterial capabilities with 0.06 and 0.08 at the dry and wet site respectively, fungal enzymatic functions were linked to a higher extent with values of 0.28 and 0.19. Most enzymatic capabilities of the fungi were positively associated (Table 2) permitting the simultaneous exploitation of different energy sources and, thus, the substitution of target substrates when they are present. Only the degradation potential for readily available xylan was negatively correlated with that of polygalacturonic acid at the wet site. When only a small amount of litter remained, a high pectinase capacity of fungi was detected.

The active fungi were isolated on two media (Rosenbrock et al., 1995). MEA is a broad-spectrum medium favouring fast-growing fungi, whereas CSEA is a nutrient poor medium with specific growth factors allowing the isolation of slow growing strains. It is, therefore, not surprising that the two isolation media produced partially different results. The respective physiological potentials were correlated to four and six of the nine groups at the dry and wet site (data not shown). In our approach, the data of the two media was combined in order to sum up a broad spectrum of the mycofloras present in the litter and to consider shifts of the isolation efficiency from MEA to CSEA minimising technique-dependent conclusions (Frankland, 1998). Isolation efficiency differed between the two media particularly during the first 6 months at the wet site. More correlations between fungal capabilities isolated on CSEA indicate that the organisms isolated on this medium exhibited broader enzymatic potentials. Noteworthy is that the ratio between isolates on CSEA and on MEA increased during the course of decomposition (data not shown). This effect was most pronounced during the first 6 months at the 'rapid-decomposing' wet site, demonstrating that MEA applies primarily to zymogenous organisms that are easy to isolate, have adapted to readily decomposable organic substances and occur predominately during the early decomposition stages whereas CSEA refers to autochthonous organisms that are difficult to isolate, have adapted to degrade indigenous stable substances and occur later. Furthermore, the higher frequency of sterile mycelia with proceeding decomposition concurs with findings of Atlas and Bartha (1998) and may be the result of a more complicated life cycle of the organisms with progressive decomposition.

At the dry site, we observed four positive and two negative correlations considering MEA, 17 positive and none negative for CSEA and ten positive and none negative for the sum of both MEA and CSEA. At the wet site, we found four positive and two negative correlations considering MEA, 11 positive and one negative considering CSEA and seven positive and one negative considering the sum of both MEA and CSEA. Consequently, the connectance between the physiological capabilities of fungal communities appears to be higher for fungi isolated from the dry site and for those isolated on CSEA that are soil-specific substrates.

Table 2
Spearman rank order correlation coefficients between physiological capabilities of fungal communities during decomposition of leaf litter at two sites in the alder forest of the Bornhöved Lake district ($n = 12$ [samplings], $p < 0.05$). Abbreviations: lipolytic lip. degradation capacity for polygalacturonic acid pol. pectolytic pec. cellulolytic cel. non-specific lignolytic lig. proteolytic pro. xylanolytic xyl

		Lip	Pol	Pec	Cel	Lig
Dry site	Pro	0.87		0.83	0.66	0.70
	Lip			0.74	0.66	0.79
	Pec				0.80	0.74
	Cel					0.80
Wet site	Pro	0.84		0.95		0.95
	Lip			0.81		0.82
	Pec				0.66	0.93
	Xyl		−0.83			

3.3. Interactions between bacteria and fungi

Fig. 1 illustrates corresponding physiological capabilities of bacterial and fungal communities. Positive correlations generally suggest that the bacterial and fungal communities compete for this nutrient pool, whereas negative correlations, as observed for lipase at the two sites, indicate shifts in importance of bacteria and fungi for the degradation of the concerned compounds during different stages of the decomposition process. In fact, other physiological capabilities neither changed in parallel nor showed any interrelation during the entire period of 12 months. However, coincidences were apparent for selected periods. For example, proteolytic capabilities changed simultaneously for bacterial and fungal communities during the first 7 months at the dry site; highest fungal proteolytic capacities occurred during the first and highest bacterial proteolytic numbers were present during the second part of the experiment; cellulolytic capabilities decreased for both bacterial and fungal communities at the two sites in summer and were lower during the second part of the experiment.

Examining the links between bacterial and fungal capacities (Table 3), cellulolytic bacteria were positively correlated with the lipolytic fungal potential. The positive connection between amylolytic bacterial density and cellulolytic and also pectolytic fungal potentials at the dry site suggests that the fungal degradation of polymers was accompanied by the bacterial mineralisation. Finally, the connectance between bacterial and fungal capabilities was higher at the dry than at the wet site being 0.25 and 0.14, respectively. This connectance was higher than that existing within the bacteria, but lower than that within fungi.

The lipolytic degradation capacity of fungi was high at

Table 3
Spearman rank order correlation coefficients between physiological capabilities of bacterial and fungal communities during decomposition of leaf litter at two sites in the alder forest of the Bornhöved Lake district ($n = 12$ [samplings], $p < 0.05$). Abbreviations: bacteria b. fungi f. cellulolytic cel. lipolytic lip. amylolytic amy. degradation capacity for polygalacturonic acid pol. ammonium-oxidising amm. proteolytic pro. xylanolytic xyl. pectolytic pec. non-specific lignolytic lig

		Amy f	Pro f	Lip f	Xyl f	Pec f	Cel f	Lig f
Dry site	Cel b			0.69		0.72		0.74
	Lip b		−0.82	−0.81		−0.81		−0.73
	Amy b					0.79	0.79	
	Pol b						−0.62	
	Amm b	0.74	0.59			0.58	0.58	
Wet site	Cel b		0.74	0.85		0.72		0.77
	Lip b		−0.85	−0.85		−0.88		−0.81
	Amm b				0.65			

the beginning of the decay while bacteria appeared to increase their lipolytic capacity progressively. The lipid fraction of leaf or needle litters generally disappeared more rapidly than non-lipid fractions (Bridson, 1985; Cortez et al., 1996), though this trend may have been obscured by extensive fungal colonisation (Bridson, 1985). Lipids, thus, seemed to play a significant role in microbial growth and the development of high microbial densities. Extractable lipids were present throughout the whole experimental period (Dilly et al., 1997) but the content considerably decreased and lipid composition may have also changed. Readily available lipids may have been mineralised at the beginning of the decomposition process by zymogenous fungi (Rosenbrock et al.,1995) whereas refractory litter-derived lipids resisted this early fungal attack and have been mineralised together with microbiologically produced lipids during the late stage of decomposition by bacteria. The zymogenous fungi were probably present on the leaf surface at the tree or had rapidly grown when the leaves came in contact with the soil. In contrast, bacteria seemed to be more abundant later as they require a certain degree of breakdown and decomposition of the leaf structure. The impression is given that leaf litter decomposition proceeds continuously, starting at the leaf at the tree. This suggestion is supported by the presence of parasitic fungi on leaves both before and after litter fall (Zvyagintsev, 1994).

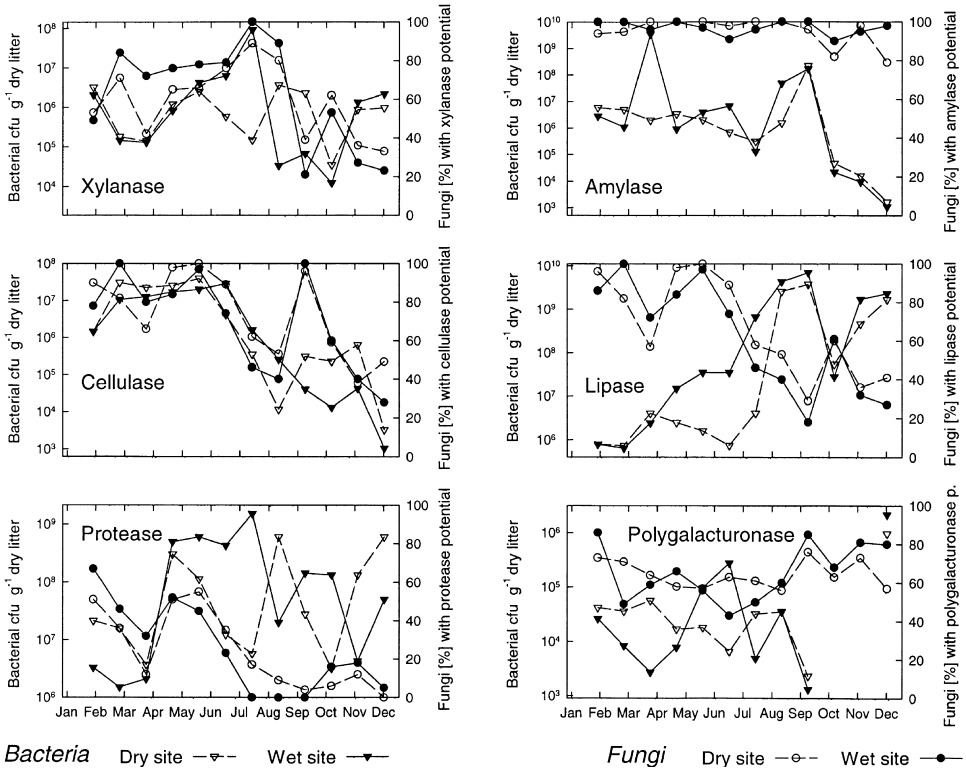

Fig. 1. Physiological capabilities of bacterial and fungal communities during decomposition of leaf litter at two sites in the alder forest of the Bornhöved Lake district.

Table 4
Best subset of bacterial and fungal capabilities which help to explain integral microbiological characteristics during decomposition of leaf litter at two sites in the alder forest of the Bornhöved Lake district ($n = 12$ [samplings], $p < 0.05$); negative signs indicate that respective components were negatively correlated with the biotic components. Abbreviations: microbial C content C_{mic}. basal respiration rate Bas. metabolic quotient qCO_2. β-glucosidase activity Glu. biomass-specific β-glucosidase activity qGlu. protease activity Pro. biomass-specific protease activity qPro. constant factor c. lipolytic lip. for others, see Table 3

	C_{mic}[a]	Bas	qCO_2	Glu	qGlu	Pro	qPro
Dry site	c	Pec f	Pec f	c	c	c	c
	-Pol b	c		Cel b	Pol b	-Xyl f	-Amy f
	Lip f	-Amy b	Cel b		Cel b	-Amy f	-Xyl f
	-Pro f	Cel f	-Lip b		[$p = 0.055$]	Pro f	Pro f
	Xyl f	Lig f	-Lig f			-Lip f	-Lip f
	Cel f		[$p = 0.066$]				
	Pec f						
Wet site	c	c	c	Pec f	c	–	c
	Cel f	Pec f	Pec f	c	Pro f		-Cel f
		-Lip f	-Lip f				
		-Lip b					

[a] Decreasing importance from upper to lower row.

3.4. Bacteria and fungi related to integral microbiological characteristics

The physiological capabilities of the bacterial and fungal communities did not provide clear explanations of the integral microbiological characteristics at the two sites. This indicates that biomass content, respiration rate and β-glucosidase and protease activity rates were not connected to the determined bacterial and fungal groups within the communities (Pennanen et al., 1999). However, the connectance between bacteria and fungi related to integral microbiological characteristics was higher than that between bacterial and fungal capabilities. This was once again higher at the dry than at the wet site being 0.30 and 0.27, respectively. The capabilities of the fungi mainly contributed to the higher connectance. The individual Spearman rank order correlation coefficients were not displayed. In Table 4, the best subset of significantly correlative physiological bacterial and fungal capabilities explaining the integral microbiological characteristics are listed. The model frequently generated a constant factor indicating that there are some background values independent from varying bacterial and fungal capabilities. In the instance of enzyme activities, this may be derived from stabilised extracellular fractions (Dilly and Nannipieri, 1998).

Overall, microbial components of the C cycle correlated more to each other than those of the N cycle. Correlations were even negative for mass and biomass-specific protease activity rate. The poor connectance may be due to restrictions of cultivation methods (Atlas and Bartha, 1998) and the fact that each method applies to another subset of the microbial communities. Consequently, the proportion of each bacterial and fungal component as part of the integral microbiological characteristics varies during decomposition of leaf litter in the regarding period especially in the N cycle.

Microbial C content was especially correlated to the cellulolytic fungal potential (Table 4). Several capabilities were additionally considered during Best Subset Regression at the dry site. Basal respiration rates and the qCO_2 corresponded to pectolytic potentials of fungi. At the dry site, the non-specific lignolytic, phenol-oxidising fungal potential concurred positively to basal respiration rate but negatively to qCO_2 suggesting that high efficiency indicated by low qCO_2 values appeared together with high lignolytic capacity. Of all C-mineralising capacities, the number of cellulolytic bacteria and the lignolytic potential of fungal isolates on CSEA were the only functions that coincided with β-glucosidase activity rate. This occurred at the dry site. At the wet site, the correlation between cellulolytic bacteria and the β-glucosidase activity rate was weaker ($p = 0.065$). Few correlations were detected in the case of the casein-hydrolysing protease activity rate. Proteolytic fungal capacities varied simultaneously with casein-hydrolysing activity rate at the dry site suggesting that fungi contributed mainly to the 'total' proteolytic capabilities here. The negative correlations between protease and capabilities of the C cycle suggest shifts in C and N enzymatic capabilities during leaf litter decomposition. At the dry site, the qPro fitted negatively to ammonium-oxidising bacterial numbers (not shown) suggesting that low N mineralisation occurred when high proteolytic capacity with reference to biomass was present.

Relating copiotrophic bacterial numbers and microbial biomass content (Fig. 2), fungi dominated the microbial biomass at the early stages of litter degradation as already observed by Swift (1976). This concurs with findings of Neely et al. (1991), of Zhang and Zak (1998) and of Mamilov et al. (2001) in which fungi were the dominant decomposers of surface residues. Consequently, the fungal communities seem preferentially to decompose the fresh leaf substrates and bacteria ensure the complete mineralisation as for N to the form of nitrate. This may also be

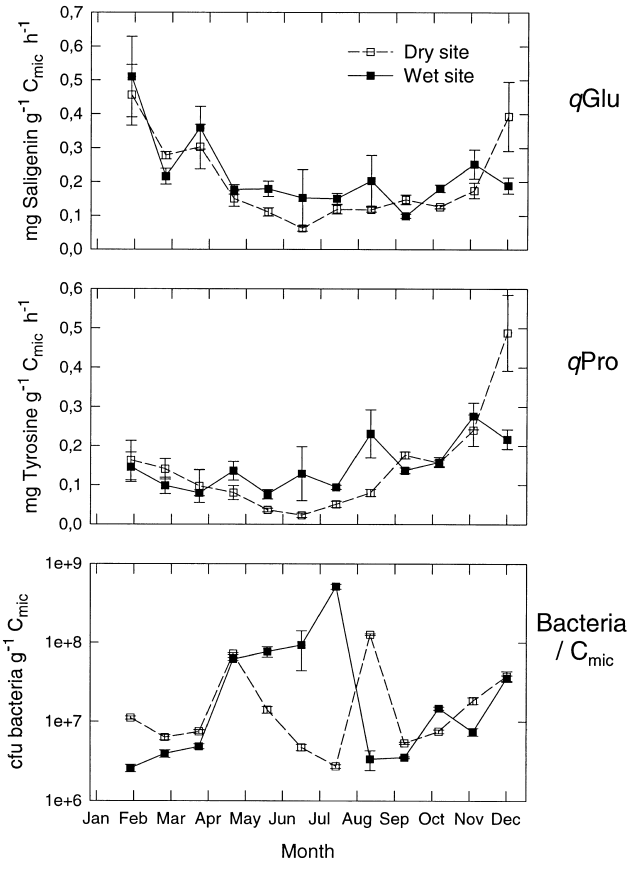

Fig. 2. Biomass-specific β-glucosidase (*q*Glu) and protease (*q*Pro) activities, and copiotrophic bacterial numbers related to microbial biomass content (Bacteria/C_{mic}) during decomposition of leaf litter at two sites in the alder forest of the Bornhöved Lake district (bars indicate standard deviations).

concluded from the correlations between cellulolytic fungal potential and amylolytic bacterial numbers. The highly specific metabolic pathways accessible to the bacteria kingdom support the hypothesis since they are particularly present under unfavourable nutritional conditions and environmental constraints.

3.5. Abiotic factors controlling the microbiological characteristics

Abiotic conditions did not affect the physiological capabilities of bacteria and fungi to the same extent as the integral microbiological characteristics like microbial C content, microbial respiration rate, and enzyme activity rates (Table 5). The connectance to abiotic factors was 0.51 and 0.38 for integral microbiological characteristics, 0.16 and 0.16 for the bacterial and 0.13 and 0.31 for the fungal enzymatic capabilities at the dry and wet site respectively. Thus, integral microbiological characteristics were apparently more dependent than the sub-components on abiotic environmental conditions. High temperature frequently reduced microbiological characteristics, and high water content generally stimulated them. The complicated interdependence was reflected by increasing correlation coefficients when abiotic properties were combined in multiple linear regression (data not shown). But, considering that temperature was highly negatively correlated with β-glucosidase activity rate in simple correlations analysis, it did not lead notably to a higher correlation coefficient in multiple linear regressions when combined with water content. Enzyme activity rates and microbial C use inefficiency (Dilly and Munch, 1998) or C availability (Cheng et al., 1996) indicated by the qCO_2, were higher at low temperature and high water level.

Variations in air humidity, pH value, mass remaining in the bags at the sampling time, organic C content and the content of Folin-reactive compounds in the litter explained some modifications of physiological capabilities and integral microbial characteristics. Folin determines the phenolic groups and is used, like ninhydrin, to estimate the amino acid content when estimating casein-hydolysing protease activity (Ladd and Butler, 1972). Variations in air humidity were negatively associated to changes of ammonium-oxidising bacterial numbers at the two sites, to microbial C content related to organic C content at the dry site and to copiotrophic and proteolytic bacteria and also to copiotrophic bacteria related to microbial C content at the wet site. Air humidity corresponded positively to protease activity rate either related to dry litter or to microbial biomass at the two sites and to degradation capacity for polygalacturonic acid of fungi at the wet site. Although there is little information on the role of air humidity to explain microbiological features in natural environments, it can be assumed that the accessible water in the air contributed to microbial processes particularly in such unprotected compartments like litter horizons (Nagy and Macauley, 1982; Pillers and Stuart, 1993). The litter underwent dramatic fluctuations in moisture (Dilly and Munch, 1996) and periodic drying is a critical limitation to microbial growth (Dix, 1984).

The amount of litter mass remaining in the bags at the sampling fitted positively with cellulolytic bacterial numbers and pectolytic fungal potential, basal respiration rate and qCO_2; negatively with lipolytic bacteria at the two sites. Pancholy and Rice (1973) concurrently indicated that cellulose-decomposing organisms were more active in the early stages of succession. With advancing decomposition states, ammonium-oxidising bacterial numbers and specific lignolytic fungal potential decreased at the dry site, as did proteolytic and lipolytic fungal potentials at the wet site. The bacterial and fungal capacity for degrading N polymers and monomers coincided to the 'integral' proteolytic activity but were differently regulated. Decreasing lignolytic potential with successive decomposition was surprising because lignin, as a refractory compound, was suggested to become relatively enriched and, thus, to degrade extensively during later stages of decomposition. Such an increase during the decomposition was observed, however, for the degradation capacity for polygalacturonic

Table 5
Best subset of bacterial and fungal capabilities and also integral microbiological characteristics related to the abiotic environmental conditions during decomposition of leaf litter at two sites in the alder forest of the Bornhöved Lake district ($n = 12$ [samplings], $p < 0.05$); negative signs indicate that respective components were negatively correlated with the abiotic components. Abbreviations: Temperature T. water content WC. air humidity AH. pH (H_2O) value pH. mass remaining MR. content of Folin-reactive compounds FRC. constant factor c. unspecific lignolytic lig*. specific lignolytic lig**. nitrite-oxidising nit. copiotrophic cop. amylolytic amy. for others, see Tables 3 and 4

	T*[a]	WC	AH	pH	MR	C_{org}	FRC
Dry site	c	Pro	c	–	Bas	c	Glu
	-*q*Glu	*q*Glu	Pro		c	Cel. b	
	-qCO_2	Glu	*q*Pro		Lig** f	C_{mic}	
	-Pro	-*q*Pro	Amm b		Amm b	C_{mic} C_{org}^{-1}	
		Pol b			-Lip b	*q*Glu	
		Amy f				-Glu	
		Xyl f					
Wet site	c	*q*Glu	c	c	Lip f	c	qCO_2
	-qCO_2	c	Pol f	-Glu	Pol f	qCO_2	Bas
	Amm b	-Xyl f	Cop b		c		Pec f
	Nitr b	-Pro b	-Pro b		Bas		
	-Bas	Pol f			Pro f		
	-*q*Glu	Pec f			Pec f		
	Glu	Cop b C_{mic}^{-1}					
	Prof	Amm b					

[a] Decreasing importance from upper to lower row.

acid of fungi and biomass-specific protease activity rate at the wet site.

Organic C content varied only slightly during the experiment (Dilly and Munch, 1996) and was positively related to cellulolytic bacteria and β-glucosidase activity rate at the two sites, to degradation capacity for polygalacturonic acid of bacteria, microbial C content, basal respiration rate and biomass-specific β-glucosidase activity rate at the dry site and to qCO_2 at the wet site. It was negatively correlated to lipolytic bacteria at the wet site. Variations of pH-values seem only to have affected β-glucosidase and biomass-specific glucosidase activity rate at the wet site. The content of Folin-reactive compounds in the litter coincided with many microbiological components, i.e., pectolytic fungal potential, basal respiration rate, qCO_2 and β-glucosidase activity rate at the two sites. At the wet site this component was additionally linked to alterations in cellulolytic and lipolytic (negatively) bacteria and also proteolytic, lipolytic, cellulolytic and non-specific lignolytic fungal potential and the microbial C content related to dry litter and organic C content. The numerous correlations between microbiological components and Folin-reactive compounds suggest that these compounds may have an important impact on, and an indicative role in the nutritional state of the microbiota.

Finally, the physiological capabilities of bacterial and fungal communities seemed to be affected by fresh litter indicated by peaking values of fungal lipolytic potential and corresponding low values of lipolytic bacteria during litter fall in September (Fig. 1).

3.6. Comparison of the sites

Abiotic factors affected, site-specifically, the microbial eco-physiology in the litter. At the dry site, variations of the microbial C content were related to shifts in bacterial and fungal capabilities (Table 4) and the biomass-specific enzyme activity rates indicating the microbial enzymatic capacities were largely reduced in summer (Fig. 3). Furthermore, bacterial and fungal cellulolytic potential decreased in summer being more reduced at the dry than at the wet site for the fungi. In contrast, the bacterial/C_{mic}-ratio showed maximal values at the wet site.

The connectance between the physiological capabilities and the integral microbiological characteristics was generally higher at the dry than at the wet site. The only exception

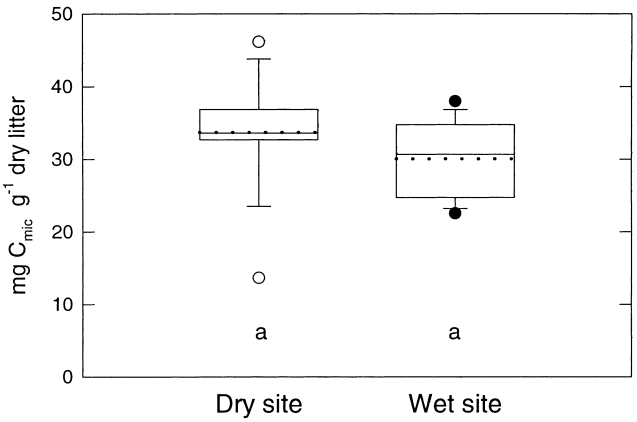

Fig. 3. Mean C_{mic} content during decomposition of leaf litter at two sites in the alder forest of the Bornhöved Lake district ($n = 12$ [samplings]; different letters indicate significant differences when applying the Mann-Whitney Rank Sum Test, $p < 0.05$; boxes encompass 25 and 75% quartiles, the central and the broken lines represent the median and the mean respectively, bars extend to the 90% confidence limits and circles show data outside the 10th and 90th percentiles).

was the connectance between fungal enzymatic capabilities and abiotic factors indicating that the fungal components respond more sensitively at the wet than at the dry site. The lower connectance at the wet site suggested that the faster decomposition process generated uncoupled microbial capabilities for the degradation of litter constituents. This may be derived from the initial rapid litter decomposition at the wet site resulting in a depletion of substrate (Dilly et al., 1997).

Since litter decomposition preceded more rapidly at the wet site than at the dry site with respectively approximately 20 and 40% of the initial mass remaining after 12 months (Dilly and Munch, 1996), the variation in biotic capabilities may be more evident there. The higher decomposition velocity at the wet site can be attributed to more favourable eutric conditions in the Histosol including pH value, water and nutrient supply near the adjacent eutrophic lake and the abundant modulating endogeous earthworms (Dilly and Irmler, 1998). Higher root densities may also have accelerated the decomposition process (Dilly et al., 1999). Although there were no significant differences in microbial C content at the two sites (Fig. 3), higher variations of the microbial biomass content at the dry site were possibly caused by higher variability of abiotic conditions such as water supply and by the lower predatory pressure of the soil fauna (Elliott, 1997; Frankland, 1998). It is, however, essential to add that microbiological data was gathered here under optimal conditions and represent potentials rather than field activities. Basal respiration rate reflects the non-water restricted mineralisation status at 22°C and, therefore, microorganisms have certainly suffered from water restriction in summer particularly at the dry site (Dilly et al., 1999).

4. Conclusions

1. Distinct interactions between bacteria and fungi during leaf litter decomposition in a black alder forest could be determined according to correlations between the physiological capabilities.
2. Fungal enzymatic potentials were generally positively linked with each other and with the corresponding physiological capabilities of bacteria suggesting a competition and a mutualistic and associative operation of bacterial and fungal communities for the degradation of litter constituents.
3. The numbers of lipolytic bacteria and the corresponding potential of fungi were negatively correlated indicating that lipolytic potential of bacteria and fungi were not present simultaneously and the importance of bacteria and fungi for lipid degradation shifted during litter decomposition.
4. The connectance between integral microbiological characteristics and physiological capabilities of bacteria and fungi was generally poor. This reflects that each method refers to another subset of the microbiota, which individually and variably contribute to the microbial biomass and the integral microbial eco-physiology during decomposition.
5. Abiotic factors generally affected integral microbiological characteristics to a higher extent than physiological capabilities of bacteria and fungi. High water content and low temperature generally stimulates microbiological potentials such as qCO_2.
6. Similar correlations were detected at the dry and wet site of the alder forest. Site-specific differences could be achieved i.e. for biomass-specific activities such as respiration rate and enzyme activity rates. The connectance indicated that the link between considered components was generally higher at the dry that at the wet site.

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References

Anderson, J.P.E., Domsch, K.-H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10, 215–221.

Atlas, R.M., Bartha, R., 1998. Microbial Ecology. Fundamentals and Applications. Addison-Wesley Publishing Company, Reading.

Bavendamm, W., 1928. Über das Vorkommen und den Nachweis von Oxidasen bei holzzerstörenden Pilzen. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 28, 257–279.

Bieleit, G., 1990. Einfluβ assoziierender Mikroorganismen auf die symbiontische Wechselwirkung zwischen *Rhizobium leguminosarum* biovar trifolii and *Trifolium repens* L. unter besonderer Berücksichtigung von *Klebsiella oxytoca*. Dissertation, Universität Hannover.

Bridson, J.N., 1985. Lipid fraction in forest litter: early stages of decomposition. Soil Biology & Biochemistry 17, 285–290.

Cheng, W., Zhang, Q., Coleman, D.C., Carroll, C.R., Hoffmann, C.A., 1996. Is available carbon limiting microbial respiration in the rhizosphere? Soil Biology & Biochemistry 28, 1283–1288.

Cohen, J.E., Briand, F., Newman, C.M., 1990. Community Food Web. Data and Theory. Springer, Berlin, p. 308.

Cortez, J., Demard, J.M., Bottner, P., Monrozier, L.J., 1996. Decomposition of Mediterranean leaf litters: A microcosm experiment investigating relationships between decomposition rates and litter quality. Soil Biology & Biochemistry 28, 443–452.

Dilly, O., Irmler, U., 1998. Succession of the food web during the decomposition of leaf litter in a black alder (*Alnus glutinosa* (Gaertn.) L.) forest. Pedobiologia 42, 109–123.

Dilly, O., Munch, J.-C., 1996. Microbial biomass content, basal respiration and enzyme litter in a black alder (*Alnus glutinosa* (L.) Gaertn.) forest activities during the course of decomposition of leaf. Soil Biology & Biochemistry 28, 1073–1081.

Dilly, O., Munch, J.-C., 1998. Ratios between estimates of microbial

biomass content and microbial activity in soils. Biology and Fertility of Soils 27, 374–379.

Dilly, O., Nannipieri, P., 1998. Intracellular and extracellular enzyme activity in soil with reference to elemental cycling. Zeitschrift für Pflanzenernährung und Bodenkunde 161, 243–248.

Dilly, O., Wachendorf, C., Irmler, U., Blume, H.-P., Munch, J.-C., 1997. Changes of abiotic and biotic parameters in the course of decomposition of leaf litter in a black alder (*Alnus glutinosa* (L.) Gaertn.) forest of a moranic landscape in Northern Germany. EcoSys 6, 31–42.

Dilly, O., Bach, H.-J., Blume, H.-P., Buscot, F., Dittert, K., Kappen, L., Kutsch, W.L., Middelhoff, U., Mogge, B., Pritsch, K., Wötzel, J., Munch, J.-C., 1999. Microbial processes and features of the microbiota in Histosols from a black alder (*Alnus glutinosa* (L.) Gaertn.) forest. Geomicrobiology Journal 16, 65–78.

Dix, N.J., 1984. Moisture content and water potential of abscised leaves in relation to decay. Soil Biology & Biochemistry 16, 367–370.

Durrands, P.K., Cooper, R.M., 1988. Development and analysis of pectic screening media for use in the detection of pectinase mutants. Applied Microdbiology and Biotechnology 28, 463–467.

Elliott, E.T., 1997. Rationale for developing bioindicators of soil health. In: Pankhurst, C., Doube, B.M., Gupta, V.V.S.R. (Eds.). Biological Indicators of Soil Health. CAB International, Wallingford, pp. 49–78.

FAO, 1988. Soil map of the world. Revised legend. World soil resources report 60, FAO, Rome.

Frankland, J.C., 1998. Fungal succession—unravelling the unpredictable. Mycological Research 102, 1–15.

Hankin, L., Anagnostakis, S.L., 1975. The use of solid media for detection of enzyme production by fungi. Mycologia 67, 598–607.

Hoffmann, G., Dedeken, M., 1965. Eine Methode zur colorimetrischen Bestimmung der β-Glucosidase-Aktivität in Böden. Zeitschrift für Pflanzenernährung, Düngung und Bodenkunde 108, 193–198.

Kjøller, A., Struwe, S., 1980. Microfungi of decomposing red alder leaves and their substrate utilization. Soil Biology & Biochemistry 12, 425–431.

Kjøller, A., Struwe, S., Bestberg, K., 1985. Bacterial dynamics during the decomposition of alder litter. Soil Biology & Biochemistry 17, 463–468.

Kreisel, H., Schauer, F., 1987. Methoden Des Mykologischen Laboratoriums. Gustav Fischer Verlag, Stuttgart.

Ladd, J., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivates as substrates. Soil Biology & Biochemistry 4, 19–30.

Mamilov, A.Sh., Byzov, B.A., Zvyagintsev, D.G., Dilly, O.M., 2001. Predation on fungal and bacterial biomass in a soddy-podzolic soil amended with starch, wheat straw and alfalfa meal. Appl. Soil Ecol. 16, 131–139.

Moore, J.C., Hunt, H.W., 1988. Resource compartimentation and the stability of real ecosystems. Nature 333, 261–263.

Nagy, L.A., Macauley, B.J., 1982. Eucalyptus leaf-litter decomposition: effects of relative humidity and substrate moisture content. Soil Biology & Biochemistry 14, 233–236.

Näveke, R., Tepper, K.P., 1982. Einführung in die Mikrobiologischen Arbeitsmethoden. Gustav Fischer Verlag, Stuttgart.

Neely, C.L., Beare, M.H., Hargrove, W.L., Coleman, D.C., 1991. Relationship between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. Soil Biology & Biochemistry 23, 947–954.

Niku-Paavola, M.L., Raaka, L., Itävaara, M., 1990. Detection of white rot fungi by a non toxic strain. Mycological Research 94, 27–31.

Omelianski, W., 1902. Über die Gärung der Zellulose. Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten Abteilung, 2, 8:193.

Pancholy, S.K., Rice, E.L., 1973. Soil enzymes in relation to old field succession: Amylase, cellulase, invertase, dehydrogenase, and urease. Journal of Soil Science Society of America 37, 47–50.

Pennanen, T., Liski, J., Baath, E., Kitunen, V., Uotila, J., Westman, C.J., Fritze, H., 1999. Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. Microbial Ecology 38, 168–179.

Pickett, S.T.A., Kolasa, J., Jones, C.G., 1994. Ecological Understanding. The Nature of Theory and the Theory of Nature. Academic Press, San Diego, p. 206.

Pillers, M.D., Stuart, J.D., 1993. Leaf-litter accretion and decomposition in interior and coastal old-growth redwood stands. Canadian Journal of Forest Research 23, 552–557.

Rosenbrock, P., Buscot, F., Munch, J.C., 1995. Fungal succession and changes in the fungal degradation potential during the initial stage of litter decomposition in a black alder forest [*Alnus glutinosa* (L.) Gaertn.]. European Journal of Soil Biology 31, 1–11.

Schinner, F., von Mersi, W., 1990. Xylanase-CM-Cellulase- and Invertase activity in soil: an improved method. Soil Biology & Biochemistry 22, 511–515.

Smith, R.E., 1977. Rapid tube test for detecting fungal cellulase production. Applied and Environmental Microbiology 34, 43–54.

Soil Survey Staff, 1988. Keys of Soil Taxonomy (fourth printing). SMSS Technical Monograph No.6. Cornell University, Ithaca, New York.

Swift, M.J., 1976. Species diversity and the structure of microbial communities in terrestrial habitats. In: Anderson, J.M., Macfadgen, A. (Eds.). The Role of Terrestrial and Aquatic Organisms in Decomposition Processes. Blackwell Scientific, Oxford, pp. 185–222.

Swift, M.J., 1982. Microbial succession during the decomposition of organic matter. In: Burns, R.G., Slater, J.H. (Eds.). Experimental Microbial Ecology. Blackwell Scientific Publications, Oxford, pp. 164–177.

Waid, J.S., 1997. Metabiotic interactions in plant litter systems. In: Cadisch, G., Giller, K.E. (Eds.). Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, pp. 145–153.

Wollum, A.G., 1982. Cultural methods for soil microorganisms. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Inc. and Soil Science Society of America, Inc, Madison, Wisconsin USA, pp. 781–801.

Zhang, Q., Zak, J.C., 1998. Potential physiological activities of fungi and bacteria in relation to plant litter decomposition along a gap size gradient in a natural subtropical forest. Microbial Ecology 35, 172–179.

Zvyagintsev, D.G., 1994. Vertical distribution of microbial communities in soils. In: Ritz, K., Dighton, J., Giller, K.E. (Eds.). Beyond the Biomass. Wiley and Blackwell Scientific Publications, Chichester and Oxford, pp. 29–37.