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Anaerobic activities of bacteria and fungi in moderately acidic conifer and deciduous leaf litter

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Abstract

The litter layer of forest soils harbors high amounts of labile organic matter, and anaerobic decomposition processes can be initiated when oxygen is consumed more rapidly than it is supplied by diffusion. In this study, two adjacent moderately acidic forest sites, a spruce and a beech–oak forest, were selected to compare the anaerobic bacterial and fungal activities and populations of conifer and deciduous leaf litter. Most probable number (MPN) estimates of general heterotrophic aerobes and anaerobes from conifer litter equaled those from deciduous leaf litter. H_2, ethanol, formate, and lactate were initially produced with similar rates in both anoxic conifer and deciduous leaf litter microcosms. These products were rapidly consumed in deciduous leaf but not in conifer litter microcosms. Supplemental ethanol and H_2 were consumed only by deciduous leaf litter and yielded additional amounts of acetate in stoichiometries indicative of ethanol- or H_2-dependent acetogenesis. The negligible turnover of primary fermentation products in conifer litter might be due to the low numbers of acetogens and secondary fermenters present in conifer litter compared to deciduous leaf litter. Fungi capable of anaerobic growth made up only 0.01–0.1% of the total anaerobic microorganisms cultured from conifer and deciduous leaf litter, respectively. Metabolic product profiles obtained from the highest anoxic, growth-positive MPN dilutions supplemented with antibacterial agents indicated that the dominant population of fungi, apparently mainly yeast-like cells, produced H_2, ethanol, acetate, and lactate both in conifer and deciduous leaf litter. Thus, despite acidic conditions, bacteria appear to dominate in the decomposition of carbon in anoxic microsites of both conifer and deciduous leaf litter.

Keywords: Anaerobic litter decomposition; Fungus; Acetate; Acetogenesis

1. Introduction

The structure of the soil microbial community involved in the decomposition of organic matter in forest ecosystems is influenced by the amount and quality of litter input dependent on the plant species composition [1,2]. Litter derived from coniferous trees, like spruce, generally displays a moderately acidic or acidic pH [3]. Since fungi seem to be more tolerant to low pH conditions than bacteria [4–6], conifer litter is thought to be predominantly decomposed by fungi under oxic conditions [7,8]. Although the litter layer of well-drained forest soils is exposed to air, some parts of the litter are oxygen-limited. Steep oxygen gradients ranging from 100 to 0% oxygen saturation within a few micrometers have been measured in the litter layer of Douglas pine [9]. These anoxic zones might be caused by the high oxygen-consuming activity in the litter layer due to the high amount of labile organic carbon. When the microbial respiration rate exceeds the supply of oxygen by diffusion into the soil, depletion of oxygen is also induced in predominantly oxic environments [10–12]. Since the diffusion of oxygen is negatively affected by a high soil water content, due to the lower diffusion coefficient of oxygen in water than in air, rainfall stimulates anaerobic processes [13,14]. Thus, anaerobic activities of fungi might also play a role in the decomposition of acidic litter subjected to anoxic conditions similar to carbon turnover processes under oxic conditions. In addition, the different substrate quality of conifer litter compared to deciduous leaf litter might affect the microbial diversity of the anaerobic microbial populations involved in the turnover of forest soil organic carbon.

Different studies conducted with pH-neutral beech litter

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demonstrate that low molecular mass organic acids (LMMOAs) and alcohols are spontaneously formed as soon as anoxic conditions are induced indicating active anaerobic microorganisms in the litter [15,16]. Acetate is the dominant organic acid detected in forest floor solutions and soil extracts [17–21]. The initial anaerobic activity is dominated by facultative anaerobic bacteria followed by the activity of obligate anaerobes like acetogens and secondary fermenters [22]. Supplemental H_2 and formate are consumed by beech litter and yield additional amounts of acetate in ratios indicative of H_2- or formate-dependent acetogenesis [15,16]. In general, methanogenic activities in oxic soils and litter are negligible [23–28]. However, in acidic peat bogs, supplemental H_2 stimulates methanogenesis and not acetogenesis [29]. Thus, methanogenesis might be also favored in the litter layer as an electron-accepting process for the oxidation of carbon derived from acidic litter. The objectives of this study were to (i) enumerate fungi and bacteria under anoxic conditions in moderately acidic litter, and (ii) study the effect of litter quality on anaerobic microbial activities and capacities of conifer and deciduous leaf litter.

2. Materials and methods

2.1. Sampling sites

Litter samples were obtained from a spruce (*Picea abies* L.) and an adjacent beech and oak (*Fagus sylvatica* L., 80%; *Quercus petraeae* L., 20%) forest on sandstone located in the Steigerwald forest in east-central Germany. Soils from both sites were cambisols (FAO classification) with a sandy loamy texture and a moder humus type. Samples were taken from April to October 1998. The top litter and soil layers (L and A horizon, respectively) were collected in plastic bags, transported refrigerated to the laboratory, and utilized within 24 h.

2.2. Analysis of soil and litter

The pHs of litter (L horizon) and mineral soil (A horizon) from spruce and beech forest sites were determined as previously described [15] and averaged 5.0 and 5.1 for the litter and 4.7 and 4.3 for the mineral soil, respectively. Dry weights of the litter samples were obtained by weighing before and after drying at 60°C for 4 days. For the analysis of the total carbon (C_{tot}) and the total nitrogen (N_{tot}) contents of the litter, dried samples were homogenized in a swing mill (MM2, Retsch, Germany), and C_{tot} and N_{tot} were measured with an element analyzer (CHN-O-rapid, Foss Heraeus, Hanau, Germany). C_{tot}, N_{tot}, and the C/N ratio in the conifer litter averaged 457.6 (mg g^{-1}), 17.4 (mg g^{-1}) and 26.5, respectively. C_{tot}, N_{tot}, and the C/N ratio in the deciduous deciduous leaf litter were 445.2 (mg g^{-1}), 16.5 (mg g^{-1}) and 27.0, respectively. Those were the means of five replicates.

2.3. Anoxic litter microcosms

For microcosm studies, 10 g dry weight of conifer or deciduous leaf litter were filled under sterile conditions into 500-ml infusion bottles (Merck ABS, Dietikon, Switzerland). To establish anoxic conditions, bottles were evacuated and flushed with N_2 three times. To study the initial anaerobic microbial activities, 50 ml (ratio 1:5; w:v) of N_2-gassed, anoxic, sterile, deionized water was added to litter microcosms inside an anaerobic chamber (Megaplex, Grenchen, Switzerland; 100% N_2 gas phase). To study the long-term anaerobic microbial capacities, 80 ml (ratio 1:8; w:v) of N_2-gassed, anoxic, sterile, deionized water was added to ensure liquid samplings during 71 days of incubation. Bottles were closed with rubber stoppers and screw-cap seals. The initial gas phase was either N_2 (100 vol%) or a mixture of N_2, H_2 and CO_2 (72:20:8 vol%) with an initial overpressure of 25–30 kPa at room temperature. Substrates were added as sterile gas or from anoxic, sterile stock solutions. Anoxic, sterile, deionized water was added to controls. Microcosms were incubated horizontally in the dark at 15°C in three replicates. The data are expressed as means ± S.D.

2.4. Cultivation media

Anoxic undefined medium (UM_{anoxic}) was prepared with a modified Hungate technique [30] and contained in mg l^{-1}: $NaHCO_3$, 7500; KH_2PO_4, 500; $MgCl_2 \cdot 6H_2O$, 50; NaCl, 400; NH_4Cl, 400; $CaCl_2 \cdot 2H_2O$, 10; yeast extract, 1000; trace element solution [31], 5.0 ml; B-vitamin solution [31], 5.0 ml. The gas phase was 100% CO_2, and the pH after autoclaving approximated 7.0. Anoxic defined medium (DM_{anoxic}) was UM_{anoxic} without yeast extract.

Anoxic tryptic soy broth (TSB_{anoxic}) (Difco Laboratories, Detroit, MI, USA) and oxic tryptic soy broth (TSB_{oxic}) did not contain dextrose and were diluted 1:10 to a final concentration of 2750 mg TSB powder l^{-1}; the pHs of both media were adjusted to 7.0 prior to autoclaving. The gas phases for TSB_{anoxic} and TSB_{oxic} were 100% N_2 and air, respectively.

Water-extractable organic matter medium (WEOM) contained an anoxic mineral solution and a concentrate of water extractable organic matter in a 8:1 (v/v) ratio. The mineral solution contained in mg l^{-1}: KH_2PO_4, 2680, K_2HPO_4, 73; $MgCl_2 \cdot 6H_2O$, 50; NaCl, 400; NH_4Cl, 400; $CuCl_2 \cdot 2H_2O$, 10. The concentrate was prepared as follows: 180 g dry weight of each conifer and deciduous leaf litter was homogenized in a mixer (Waring Commercial Blender, Bender and Holbein, Zürich, Switzerland). Another 20 g of each conifer and deciduous leaf litter were crushed with a bead beater (Bead Beater Model 1107990, Biospec Products, USA) using glass beads (0.5 mm diameter). Both homogenates were mixed, added to 4 l of deionized water, and placed on an end-over-end shaker for 16 h at 5°C. The suspension was centrifuged

(Beckman J2-HS Centrifuge, Beckman, Fullerton, CA, USA), and the supernatant was filtered through fiber glass filters, frozen in an acetone–dry-ice bath, and freeze-dried (Christ ALPHA 1-4 freeze dryer, Braun Biotech International, Germany). The dried frozen compounds were re-diluted in 400 ml deionized water and filter-sterilized into sterile infusion bottles under a 100% Ar gas phase. The final pH approximated 5.0.

For the dilution series, a mineral solution was prepared that contained in mg l^{-1}: K_2HPO_4, 225; KH_2PO_4, 225; $(NH_4)_2SO_4$, 450; NaCl, 450; $MgSO_4 \cdot 7H_2O$, 45; the solution was dispensed under a 100% CO_2 gas phase. After autoclaving, the pH approximated 6.8.

2.5. Enumeration of the anaerobic microorganisms

With the media used, numbers of cultured cells were determined by the most probable number (MPN) technique [32], as previously described [22]. All substrates and inhibitors were added from sterile stock solutions. The cultivation temperature for all enumerations was 15°C. Inoculated tubes that contained H_2 as a substrate were incubated horizontally; tubes with an oxic gas phase were incubated on a shaker (approximately 100 cycles min^{-1}). Tubes with an oxic gas phase were counted positive by measuring the optical density (OD_{660}); tubes with an anoxic gas phase were counted positive by measuring the optical density (OD_{660}) and by measuring the consumption of substrates and the formation of products like LMMOAs, alcohols or gases. Uninoculated MPN tubes were used as controls.

Neutrophilic heterotrophic aerobes and anaerobes were estimated with TSB_{oxic} and TSB_{anoxic}, respectively. H_2- and vanillate-utilizing anaerobes were determined in UM_{anoxic} by assessing the consumption of supplemental H_2 (20 vol%) or vanillate (3 mM). Inoculated tubes with UM_{anoxic} without supplemental H_2 or vanillate were used as controls to determine the microbial products from yeast extract fermentation. Ethanol- and lactate-utilizing anaerobes were determined in DM_{anoxic} by assessing the consumption of supplemental ethanol (3 mM) or lactate (3 mM). Acid-tolerant heterotrophic anaerobes were estimated with WEOM at pH 5. To differentiate between bacteria and fungi, either cycloheximide (500 μg ml^{-1} medium) or a mixture of antibacterial agents (300 μg penicillin ml^{-1}, 200 μg streptomycin ml^{-1}, and 200 μg kanamycin ml^{-1}) were added to WEOM to inhibit growth of fungi and bacteria, respectively. All tubes were incubated for 3 months.

2.6. Analytical techniques

Headspace gases were measured with Hewlett-Packard Co. (Palo Alto, CA, USA) 5980 series II gas chromatographs [26]. Concentrations were corrected for the changing liquid-to-gas phase volume ratio due to liquid (0.5 ml) sampling. Aliphatic acids, alcohols, and aromatic compounds were determined with Hewlett-Packard 1090 series II high performance liquid chromatographs [26].

3. Results

3.1. Microbial activities and capacities in anoxic conifer and deciduous leaf litter microcosms

Rainfall stimulates anaerobic microbial processes in oxic soils [13,14]. However, microsensor measurements demonstrate that organic particles in litter can be O_2-free under dry field conditions [9]. Thus, initial anaerobic microbial activities in anoxic microcosms might be similar to processes initiated in litter after rainfall, whereas anaerobic microbial capacities observed after 5–15 days might be more relevant for processes in those microsites that experience long-term anoxic conditions.

Both conifer and deciduous leaf litter spontaneously formed LMMOAs when incubated under anoxic conditions (Fig. 1A,B). The initial formation rates of acetate, ethanol and formate approximated 0.3, 0.4, and 0.2 μmol g^{-1} dry wt h^{-1} in conifer litter, respectively, and 0.4, 0.3, and 0.2 μmol g^{-1} dry wt h^{-1} in deciduous leaf litter, respectively. Lactate and succinate were detected both in conifer and in deciduous leaf litter microcosms (Fig. 1A,B), whereas H_2, malate and propionate were detected in trace amounts only in deciduous leaf litter microcosms (data not shown). Concomitantly CO_2 was formed with rates that approximated 0.9 and 1.2 μmol g^{-1} dry wt litter h^{-1}, respectively (data not shown).

In deciduous leaf litter microcosms, initially formed lactate, formate, succinate, malate, and H_2 were consumed within 15 days of incubation, and acetate and butyrate became the dominant organic products (Fig. 1D). Ethanol was consumed within 30 days of incubation. The final concentrations of acetate, butyrate, and propionate approximated 256, 211, and 53 μmol g^{-1} dry wt litter, respectively. In contrast, initially formed lactate and formate were not completely consumed in conifer litter microcosms within 71 days of incubation (Fig. 1C). In conifer litter microcosms, ethanol reached maximum concentrations of 79 μmol g^{-1} dry wt and was consumed within 62 days of incubation. The final concentrations of acetate, butyrate, and propionate approximated 180, 104, and 22 μmol g^{-1} dry wt litter. CH_4 was not detected in either conifer and deciduous leaf litter microcosms during 71 days of incubation.

3.2. Effect of supplemental ethanol and H_2 on the formation of acetate in anoxic conifer and deciduous leaf litter microcosms

Since the turnover of ethanol differed in conifer and deciduous leaf litter microcosms, ethanol was supple-

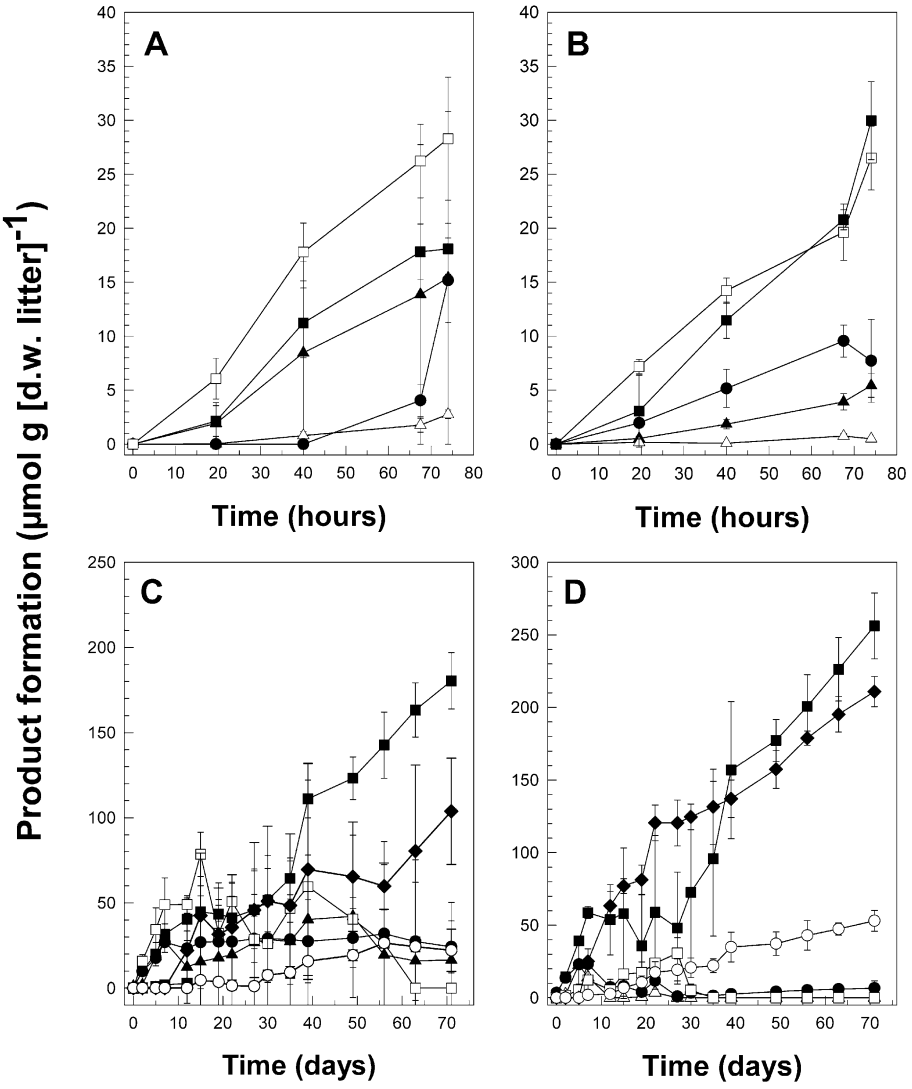

Fig. 1. Turnover of LMMOAs and alcohols during short- (A,B) and long-term (C,D) anoxic incubations of conifer (A,C) and deciduous leaf (B,D) litter. Litter was incubated at 15°C under an N_2 gas phase in a 1:5 dilution (w:v) (A,B) or in a 1:8 dilution (w:v) (C,D) with anoxic water, respectively. Data are the averages (±S.D.) of three replicates. Symbols: □, ethanol; ■, acetate; ▲, lactate; △, succinate; ●, formate; ○, propionate; ◆, butyrate.

mented to anoxic litter microcosms to study its consumption under controlled conditions. In deciduous leaf litter microcosms, the consumption of supplemental ethanol started after 5 days of incubation, and additional amounts of acetate were concomitantly produced compared to the unsupplemented control (Fig. 2B). The ratio of ethanol consumed to acetate produced approximated 1.8:3, which is close to the theoretical 2:3 ratio that is indicative of ethanol-dependent acetogenesis [33]. In contrast, only small amounts of supplemented ethanol appeared to be consumed in conifer litter microcosms, but no additional acetate was formed within 28 days of incubation (Fig. 2A).

Since acetogenic bacteria seemed to be involved in the consumption of supplemental ethanol in deciduous leaf litter microcosms, H_2 and CO_2 were supplemented to litter microcosms. In deciduous leaf litter microcosms, the consumption of supplemental H_2 started after 7 days of incubation concomitantly with the consumption of initially formed H_2 in the unsupplemented controls (data not shown). The concentration of supplemental CO_2 increased parallel to the unsupplemented controls during the first 7 days of incubation, and then decreased concomitantly with H_2 consumption. Concomitantly with the consumption of H_2 and CO_2, additional amounts of acetate were formed compared to the unsupplemented controls (data not shown). H_2 was totally consumed after 16 days of incubation. The ratio of H_2 consumed to acetate formed approximated 5.6:1, which is somewhat higher than the theoretical 4:1 ratio that is indicative of H_2-dependent acetogenesis [33,34]. In contrast, only small amounts (approximately 60 µmol g^{-1} dry wt litter) of supplemental H_2 (400 µmol g^{-1} dry wt litter) were consumed in conifer litter microcosms during 25 days of incubation, and no additional acetate was formed (data not shown).

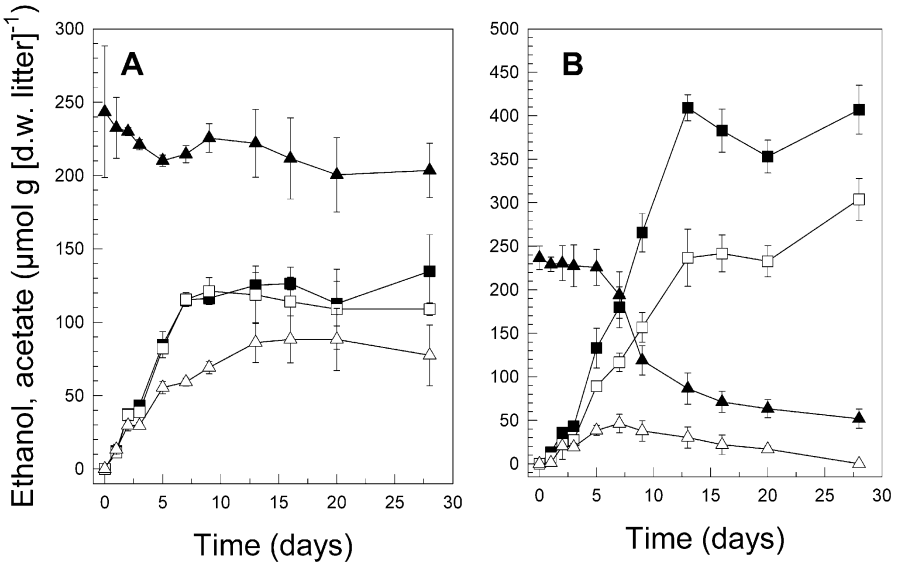

Fig. 2. Consumption of supplemented ethanol by conifer (A) and leaf (B) litter. Litter was incubated in a 1:5 dilution (w:v) with anoxic water under an N_2 gas phase at 15°C; data are the averages (±S.D.) of three replicates. Symbols: ▲, ethanol; △, ethanol formed in unsupplemented controls; ■, acetate; □, acetate formed in unsupplemented control.

3.3. Population differences of the cultured anaerobic microorganisms of conifer and deciduous leaf litter

The number of heterotrophic aerobes cultured at pH 7 approximated 10^9 g^{-1} dry wt litter both in conifer and deciduous leaf litter (Table 1). The number of heterotrophic anaerobes cultured at pH 7 approximated 10^8 g^{-1} dry wt litter in both conifer and deciduous leaf litter, whereas the numbers of heterotrophic anaerobes cultured at pH 5 in WEOM were approximately 10 times lower. The addition of an antifungal agent to WEOM did not significantly ($P < 0.05$, as determined by the Mann–Whitney U-test) affect the numbers of cultured anaerobes either in conifer or in deciduous leaf litter compared to WEOM lacking antifungal agent. However, the addition of antibacterial agents to WEOM significantly decreased the number of cultured anaerobes indicating that the population of cultured fungi capable of anaerobic growth made up only 0.01 and 0.1% of the total cultured anaerobes in conifer and in deciduous leaf litter, respectively (Table 1).

Acetate was detected in all MPN dilutions that scored positive for growth indicating that the number of total cultured anaerobes was similar to the number of acetate producers. Higher numbers of cultured ethanol-, lactate-, and H_2-utilizing anaerobes were detected in deciduous leaf litter compared to conifer litter. The consumption of supplemental ethanol yielded acetate in all positive DM_{anoxic} MPN dilutions; stoichiometries were indicative of ethanol-dependent acetogenesis. The consumption of H_2 was coincident with the production of acetate; the average H_2-to-acetate ratios in positive dilutions approximated 6.1:1. The consumption of vanillate yielded acetate and protocatechuate, catechol, and guiacol. Although most acetogens grow very poorly under H_2–CO_2 [33], numbers of cultured H_2-utilizing acetogens were higher than those of cultured vanillate-utilizing acetogens. Both in deciduous

Table 1
MPN values of different metabolic types obtained from Steigerwald conifer and deciduous leaf litter[a]

Metabolic type[b]	Medium	pH	Substrates, antibiotics	MPN (g^{-1} dry wt litter)	
				Conifer litter	Leaf litter
Heterotrophic aerobes	TSB_{oxic}	7	complex	4×10^9 (8.6×10^8–1.9×10^9)[b]	4×10^9 (8.6×10^8–1.9×10^9)
Heterotrophic anaerobes	TSB_{anoxic}	7	complex	2.3×10^8 (4.9×10^7–1.1×10^9)	4×10^8 (8.6×10^7–1.9×10^9)
Heterotrophic anaerobes	WEOM	5	complex	1.5×10^7 (3.2×10^6–7×10^7)	4×10^7 (8.6×10^6–1.9×10^8)
Heterotrophic anaerobic bacteria	WEOM	5	complex antifungal agents	9×10^6 (1.9×10^6–4.2×10^7)	9×10^7 (1.9×10^7–4.2×10^8)
Heterotrophic anaerobic fungi	WEOM	5	complex antibacterial agents	2.1×10^3 (4.5×10^2–9.8×10^3)	4×10^4 (8.6×10^3–1.9×10^5)
H_2-utilizing anaerobes	UM_{anoxic}	7	H_2–CO_2	1.5×10^2 (3.2×10^1–7×10^2)	9×10^4 (1.9×10^4–4.2×10^5)
Vanillate-utilizing anaerobes	UM_{anoxic}	7	vanillate	1.5×10^2 (3.2×10^1–7×10^2)	2.3×10^2 (4.9×10^1–1.1×10^3)
Ethanol-utilizing anaerobes	DM_{anoxic}	7	ethanol	2.3×10^1 (5–1.1×10^2)	2.3×10^3 (4.9×10^2–1.1×10^4)
Lactate-utilizing anaerobes	DM_{anoxic}	7	lactate	4×10^1 (9–1.9×10^2)	2.3×10^4 (4.9×10^3–1.1×10^5)

[a]Conifer and deciduous leaf litter (L horizon) were collected in October 1998.
[b]MPN dilutions were incubated in three replicates at 15°C for 90 days.

Table 2
Formation of organic products and H_2 in MPN dilutions of conifer and deciduous leaf litter obtained from anoxic WEOM[a]

MPN dilution	Products formed in conifer litter MPN dilutions (mM)[b]							Products formed in leaf litter MPN dilutions (mM)						
	Succinate	Lactate	H_2	Acetate	Propionate	Butyrate	Ethanol	Succinate	Lactate	H_2	Acetate	Propionate	Butyrate	Ethanol
10^{-1}	0	0	0.5 ± 0.1	3.8 ± 0.3	0.4 ± 0.2	0.7 ± 0.3	0	0	0	0.4 ± 0.2	5.6 ± 0.8	1.0 ± 0.6	1.1 ± 0.1	0
10^{-2}	0	0	1.9 ± 0.2	1.7 ± 0.6	0.1 ± 0.4	0.6 ± 0.4	0.4 ± 0.6	0	0	1.0 ± 0.2	2.9 ± 0.5	1.3 ± 0.1	0.3 ± 0.4	0
10^{-3}	0.2 ± 0.2	0.8 ± 0.4	1.9 ± 0.4	1.9 ± 0.7	0	0	0.4 ± 0.3	0	0	1.8 ± 0.3	2.8 ± 0.4	1.1 ± 0.4	0	0
10^{-4}	0.4 ± 0.1	1.1 ± 0.1	1.6 ± 0.2	2.5 ± 0.3	0	0	0.7 ± 0.5	0.2 ± 0.1	0.8 ± 0.1	1.6 ± 0.1	2.3 ± 0.3	0.2 ± 0.3	0	0
10^{-5}	0.4 ± 0.3	1.4 ± 0.1	1.8 ± 0.3	2.4 ± 0.3	0	0	0.4 ± 0.3	0.3 ± 0.1	1.2 ± 0.2	1.8 ± 0.4	2.4 ± 0.2	0	0	0
10^{-6}	0.7 ± 0.3	0.8 ± 0.4	1.3 ± 0.4	1.7 ± 0.1	0	0	0.4 ± 0.4	0.4 ± 0.2	0.4 ± 0.2	2.9 ± 0.5	2.1 ± 0.1	0	0	0
10^{-7}	0	1.2 ± 0.8	0	0.6 ± 0.5	0	0	0.2 ± 0.3	0.1 ± 0.1	0.4 ± 0.2	0	1.7 ± 0.1	0	0	0
10^{-8}	0	0	0	0.4 ± 0.7	0	0	0	0	0	0	0	0	0	0
10^{-9}	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Product profiles were determined after 90 days of incubation.
[b]Values are averages (± S.D.) of three replicates.

leaf and in conifer litter MPN series, lactate was converted to acetate and propionate indicating the activity of secondary fermenters. CH_4 was not detected in any of these MPN tubes.

3.4. Effect of antibiotics on metabolic product profiles

The composition of the anaerobic microbiota enumerated from conifer and deciduous leaf litter seemed to be affected by the source of the litter. Thus, metabolic product profiles from MPN series of WEOM at pH 5 were assessed after 90 days of incubation to determine the contribution of bacteria and fungi to the turnover of litter carbon under anoxic conditions. Product profiles of the lowest and highest dilutions are representative of the activities of the total cultured anaerobic microbiota and the dominant anaerobic microbiota, respectively. The metabolic product profiles of the lowest dilution were similar in both conifer and deciduous leaf litter WEOM MPN series (Table 2). In general, higher amounts of acetate were produced by deciduous leaf litter by all dilutions, whereas ethanol is additionally produced by higher dilutions of the conifer litter WEOM MPN series.

The addition of the antifungal agent cycloheximide to conifer and deciduous leaf litter WEOM MPN series yielded similar metabolic product profiles compared to the unsupplemented WEOM MPN series (Table 3, and data not shown for products formed in MPN series of deciduous leaf litter). However, ethanol was not detected in conifer litter MPN series supplemented with cycloheximide (Table 3). When a mixture of antibacterial agents was added to conifer and deciduous leaf litter WEOM MPN series, succinate, lactate, and ethanol were additionally detected in the lowest dilution, whereas lower amounts of acetate were produced (Table 3, and data not shown for products formed in MPN series of deciduous leaf litter). Propionate and butyrate were not produced in conifer litter MPN series supplemented with antibacterial agents (Table 3). Microscopic examinations of WEOM MPN series supplemented with antibacterial agents revealed that yeast-like cells dominated the higher dilutions in both conifer and deciduous leaf litter.

4. Discussion

In general, fungi have a wider pH tolerance than bacteria [35]. The pH optimum of cultivated aerobic soil fungi at pH 5–6 lies up to 2 units below the pH optimum of soil bacteria [6,36], and the ratio of fungal to bacterial respiration in soils increases with decreasing pH [37]. In acidic forest soils, fungi predominate the cultured microbial communities [6] and seem to be mainly involved in the aerobic decomposition of organic carbon in such soils. Facultative

Table 3
Effect of antibacterial and antifungal agents on the formation of organic products and H_2 in MPN dilutions of conifer litter obtained from anoxic WEOM[a]

MPN dilution	Products formed in conifer litter MPN dilutions supplemented with antifungal agents (mM)[b]							Products formed in conifer litter MPN dilutions supplemented with antibacterial agents (mM)						
	Succinate	Lactate	H_2	Acetate	Propionate	Butyrate	Ethanol	Succinate	Lactate	H_2	Acetate	Propionate	Butyrate	Ethanol
10^{-1}	0	0	0.5 ± 0.1	2.7 ± 0.3	0.4 ± 0.5	0.7 ± 0.1	0	0.4 ± 0.2	0.4 ± 0.1	1.6 ± 0.2	0.7 ± 0.3	0	0	0.4 ± 0.2
10^{-2}	0	0	2.0 ± 0.4	1.9 ± 0.6	0.4 ± 0.4	0	0	0.5 ± 0.2	0.6 ± 0.2	1.5 ± 0.1	1.8 ± 0.5	0	0	1.8 ± 0.8
10^{-3}	0.1 ± 0.2	0.3 ± 0.3	2.2 ± 0.2	1.6 ± 0.2	0	0	0	0.3 ± 0.3	0.5 ± 0.3	1.6 ± 0.3	0.4 ± 0.4	0	0	1.1 ± 0.5
10^{-4}	0.1 ± 0.1	0.6 ± 0.2	2.0 ± 0.2	1.1 ± 0.4	0	0	0	0	0.6 ± 0.5	1.6 ± 0.2	0.8 ± 1.0	0	0	1.2 ± 0.7
10^{-5}	0.2 ± 0.2	1.0 ± 0.1	1.8 ± 0.5	1.4 ± 0.4	0	0	0	0	0	0	0	0	0	0
10^{-6}	0	1.5 ± 0.1	1.8 ± 0.2	0.8 ± 0.6	0	0	0	0	0	0	0	0	0	0
10^{-7}	0	0.4 ± 0.8	0	0	0	0	0	0	0	0	0	0	0	0
10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Product profiles were determined after 90 days of incubation.
[b]Values are averages (± S.D.) of three replicates.

anaerobic fungi like yeasts have been isolated from soils, fruits, animals or feces [38]. Obligate anaerobic fungi have predominantly been isolated from anoxic gastrointestinal tracts of ruminant and non-ruminant herbivores, where they contribute to the digestion of cellulose, xylose, and phenolic esters [39]. In the present study, enumeration studies conducted with moderately acidic forest soils demonstrated that fungi capable of anaerobic growth made up only 0.01–0.1% of the total anaerobic microbiota cultured from conifer and deciduous leaf litter, respectively. Although MPN techniques do not assess total microbial populations [40], MPN data obtained in this study indicated that cultured fungi capable of anaerobic growth seemed to play a subordinate role in the anaerobic decomposition of litter carbon despite the low pH conditions. Metabolic product profiles obtained from the highest growth-positive dilution of WEOM supplemented with antibacterial agents indicated that the dominant population of fungi, apparently mainly yeast-like cells, was involved in the production of H_2, ethanol, acetate, and lactate in both conifer and deciduous leaf litter. Ethanol was the main organic end product detected; and the formation of ethanol is typical for various yeasts [41]. Apparently, cultured fungi capable of anaerobic growth did not have the capacity to utilize primary fermentation products, because H_2, ethanol, and lactate were also detected as end products in the lowest growth-positive dilution of WEOM supplemented with antibacterial agents.

The adjacent spruce and beech–oak forest sites were similar with respect to the geological underground, soil properties, local climate, and pH. Numbers of general heterotrophic aerobes and anaerobes cultured from conifer litter equaled those from deciduous leaf litter. Thus, the microbial colonization of the litter seemed not to be quantitatively influenced by the different litter material, if we assume that the active microbiota in conifer and deciduous leaf litter was comparatively well represented by the MPN technique. WEOM that contained in situ substrates with a pH of 5 yielded lower numbers of cultured heterotrophic anaerobes compared to the complex TSB medium adjusted to pH 7. Thus, either the indigenous microbiota capable of anaerobic growth displayed a higher pH optimum than the in situ pH, or water-extractable organic matter from conifer and deciduous leaf litter was not a suitable substrate for the majority of the litter microbiota capable of anaerobic growth.

In general, facultative anaerobes constitute about 10% of the aerobic population [10,42]. In the present study, metabolic product profiles of the highest growth-positive MPN dilutions from acidic conifer and deciduous leaf litter were indicative of the activity of mixed acid fermenters [12] similar to results obtained with pH-neutral beech litter [22]. The rapid production of mixed acid fermentation products in anoxic litter microcosms indicated that the microbiota capable of anaerobic activities in litter was adapted to oxygen fluxes, and that the initial activity seemed to be dominated by facultative anaerobic primary fermenters. Labile organic matter mobilized by moistening of litter material [43] might be utilized by the litter microbiota under anoxic conditions such as dead microbial biomass or released cell solutes including organic osmoprotectants like amino acids, quaternary ammonium compounds, glycerol, etc. [44,45].

Formation rates of primary fermentation products were similar in acidic conifer and deciduous leaf litter. However, these rates were lower than those obtained from pH-neutral deciduous leaf litter [22] indicating that the initial microbial activity of conifer and deciduous leaf litter under anoxic conditions seemed to be more affected by pH than by substrate quality. Conifer and deciduous leaf litter displayed similar organic carbon contents and C/N ratios. However, microbial biomass C, basal respiration, and cellulase activity tend to be higher in beech compared to spruce litter [46], indicating a generally higher microbial activity in beech compared to spruce litter. In this study, deciduous leaf litter also displayed higher basal respiration rates than conifer litter (Reith, personal communication). High contents of more recalcitrant waxes, lignins, resins, and phenols present in conifer litter might lower the decomposition capacities under anoxic conditions similar to oxic conditions [35,47,48].

In contrast to conifer litter microcosms, initially formed H_2, ethanol, succinate, and lactate were rapidly consumed in deciduous leaf litter microcosms after 5 days of incubation. Supplemented ethanol or H_2–CO_2 were consumed in deciduous leaf litter microcosms; and the additional production of acetate was indicative for either ethanol- or H_2–CO_2-dependent acetogenesis [33,34]. Acetogenic bacteria seem to be well adapted to variations of oxygenation in the litter layer [22,28] and can even reduce small amounts of oxygen [49]. However, acetogenic capacities of conifer litter were negligible, and numbers of cultured H_2-utilizing acetogens in conifer litter were approximately 1000 times lower than those of deciduous leaf litter. It appears that conifer litter inhibits in some way the growth of acetogens and secondary fermenters, whereas in deciduous leaf litter these groups can reach a substantial biomass and then start to influence the turnover of fermentation products. Methanogenic activities were negligible both in moderately acidic conifer and in deciduous leaf litter, although some methanogenic archaea can tolerate low pH conditions [50] and exhibit a certain degree of aerotolerance [51,52]. Long-term anoxic conditions seem to be necessary to permit methanogenesis like in acidic peat bogs [29,53–55]. The low abundance of acetogens, secondary fermenters and methanogens in conifer litter suggests that the further turnover of primary fermentation products in situ is linked to aerobic processes. Consumption under oxic conditions will occur either when oxygen diffuses back to formerly anoxic litter zones during drying periods, or when fermentation products are leached with the forest floor solution to oxic zones.

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