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Antagonism between Bacteria and Fungi on Decomposing Aquatic Plant Litter

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ABSTRACT

Bacterial and fungal decomposers of aquatic plant litter may exhibit either synergistic or antagonistic interactions, which are likely to influence microbial growth as well as the decomposition of litter and, eventually, the carbon metabolism of aquatic systems. To elucidate such interactions, we inoculated decomposing *Phragmites* culms in microcosms with fungal isolates and with natural communities of bacteria and fungi in different combinations. The development of fungal and bacterial biomass and the carbon dynamics were studied during several months of degradation. The results show a bilateral antagonistic relationship between bacteria and fungi. After 3 months, fungal biomass accumulation was approximately 12 times higher in the absence than in the presence of bacteria. Bacterial biomass accumulation was about double in the absence of fungi compared to when fungi were present. Similar interactions developed between a natural assemblage of bacteria and five different fungal strains isolated from *Phragmites* litter (three identified hyphomycetes and two unidentified strains). Despite the great difference in biomass development between the treatments, the carbon metabolism was similar regardless of whether fungi and/or bacteria were present alone or in coexistence. We suggest that the antagonism between bacteria and fungi is an important controlling factor for microbial colonization and growth on aquatic plant litter.

Introduction

Fungi are the primary degraders of plant litter in streams [2, 34]. There is increasing evidence that they play an important role in the decomposition in stagnant waters as well, e.g., marshes and littoral zones of lakes [14, 20, 21, 26, 29]. Bacteria are additional important microbial degraders of litter in lentic [12, 25, 32] as well as lotic [1] aquatic systems. The two major groups of heterotrophic osmotrophs that colonize and degrade submersed litter are phylogenetically and morphologically very different. Hence, they show differences in life form (single cells in bacteria versus hyphae in fungi) and growth characteristics. However, they contribute to the same ecosystem process, i.e., the degradation of organic matter.

The decomposition by fungi and bacteria is controlled by several factors, such as temperature [22, 32], water chemistry [31, 34], and plant litter quality [2]. However,

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the factors determining the relative importance of fungi and bacteria in degrader communities are poorly known. There is evidence for a number of different interactions between bacteria and fungi. Synergistic relationships may persist throughout the decomposition of detrital particles and may be of benefit to bacteria and/or fungi. For example, Bengtsson [3] found that both fungi and bacteria on degrading leaf litter grew faster in presence of the other group, although the mechanisms behind this remain unclear. In contrast, Wohl and McArthur [35] found an antagonistic effect from actinomycetes on growth of two aquatic fungi. Similar results were found by Møller et al. [23] in an experimental study of the interactions between bacteria and fungi on decomposing beech leaves in soil. The antagonism between the groups was suggested to be due to carbon competition. Thus, there are reasons to believe that the interactions between bacteria and fungi can be of a negative as well as a positive nature.

In a previous study of fungal and bacterial colonization of leaf litter in lakes, fungi were abundant when bacterial numbers were low, and vice versa (Denward and Tranvik, submitted). Possibly, this was due to fungal–bacterial interactions influencing the distribution of these two groups. The current study aimed to assess the occurrence of such interactions experimentally, and to evaluate their potential effects on microbial degrader biomasses and on the overall carbon metabolism. We demonstrate a general and bilateral antagonism between bacteria and fungi, which most likely was not caused solely by direct resource competition, since the presence of one group inhibited the growth of the other group long before the resources were exhausted. However, although these interactions had a crucial impact on occurrence of the major groups of degraders, their influence on major ecosystem functions such as community respiration was minor.

Methods

Experimental Overview

In order to investigate the potential role of fungal–bacterial interactions for the development of fungal and bacterial biomass, we inoculated gas-tight microcosms containing sterilized pieces of reed culms with fungi only, bacteria only, or both fungi and bacteria together. In total, 180 bottles were prepared. After 1, 2, 4, 7, 17, 31, 49, 65, 79, and 93 days, four replicate bottles of each inoculum type and two sterile control bottles were removed for analysis. Before removal of the gas-tight stopper from each sampled bottle, samples for dissolved oxygen and carbon dioxide analyses were withdrawn by syringe. Then, the rubber stopper was removed and the water and detritus were separated with a 20-μm mesh sized net. The pH was measured (for all sampling days except 1, 2, and 4) and the water was sampled for analyses of DOC (dissolved organic carbon) and bacterial biomass. After homogenization of the litter, samples for analysis of litter-associated microbial biomass were taken. Lastly, the litter was dried and analyzed for carbon and nitrogen content. Carbon turnover was estimated from data from sampling day 79. The parameters used for this calculation were fungal biomass, bacterial biomass associated with the litter and suspended in the water, carbon dioxide, DOC as well as carbon content and dry weight of remaining litter.

In a complementary experiment, we tested whether the observed fungal–bacterial interactions are specific to the fungal strain studied in the main experiment, or if they occur more generally. The development of bacterial and fungal biomass was studied in treatments with five different fungal strains in presence and absence of bacteria. Samples for analysis of litter-associated microbial biomasses were taken at experiment startup and at ending after 50 days. When not explicitly stated otherwise, the main experiment is referred to below.

Preparation of Inocula

Pieces of standing dead *Phragmites australis* (Cav.) Trin. ex Steud (common reed) were collected from beneath the ice in the littoral zone of Lake Mälaren in southeastern Sweden in March 2001. The detritus was brought to the laboratory, where it was kept in lake water at room temperature for about 1 month, in order to allow the development of bacteria and fungi on the decaying litter. Thereafter, four types of inocula were prepared. A mixed inoculum of fungi and bacteria and a fungal-free bacterial inoculum were prepared using a modified version of the protocols described by Møller et al. [23] and Fægri et al. [10]. *Phragmites* culms (20 g wet weight) were homogenized with a kitchen mixer after suspension in 200 mL of artificial lake water (15 mg anhydrous $CaCl_2$ L^{-1}, 15 mg $MgSO_4 \cdot 7H_2O$ L^{-1} and 20 mg $NaHCO_3$ L^{-1}, autoclaved for 60 min, no nutrients added) three times 1 min with 5 min of intermittent cooling on ice. The resulting suspension was centrifuged at 1000 *g* for 15 min and the supernatant was collected. The pellet was suspended in 200 mL of artificial lake water, blended in the mixer for 1 min, and centrifuged again as above. The supernatants from the two centrifugations were pooled, filtered through a 3 μm pore size membrane filter, and used as a bacterial inoculum ("Bacteria" in Table 1). The pellet was diluted again with 200 mL artificial lake water, and used as an inoculum of both fungi and bacteria ("Fungi + bacteria" in Table 1). To check for the presence of fungi in inoculum "Bacteria," aliquots of 100 μL were spread on plates containing 50 mg streptomycin L^{-1} and 50 mg ampicillin L^{-1} in Hagem agar medium (5 g glucose, 0.5 g NH_4NO_3, 0.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 5 g malt extract, 20 g agar, 1000 mL H_2O, pH 5.5). The plates showed no fungal growth after 10 days at 20°C. The presence of both fungi and bacteria in inoculum

"Fungi + bacteria" was verified in the same way but on plates without antibiotics. The inocula were kept at 10°C for 5 days, until the start of the experiment.

A natural inoculum of fungi without bacteria could not be achieved by the fractionation procedures described above. In order to get bacteria-free fungal inocula, partly decomposed *Phragmites* culms were collected from beneath ice in February 2001 at the same location as the inocula described above. Five different fungal strains were isolated and precultured on plates containing Hagem agar. The isolates include the three hyphomycetes *Alternaria alternata* (Fries:Fries) von Keissler, *Epicoccum nigrum* Link, and *Phialocephala* sp. and two unidentifiable species (CBS Identification Services, The Netherlands), denoted strain I and strain II (in the main experiment strain I is referred to as Cult F). The *Phialocephala* strain may be a species not yet described and is currently referred to as CBS 110456 in the CBS collection.

Startup

Pieces of *Phragmites* culms were collected from above ice in January 2001. The litter was dried, and stored at room temperature. Prior to the experiment start, the culms were heated at 90°C for 3 days. Preliminary tests showed that this procedure efficiently degraded ergosterol associated with the litter. In early May 2001, culm pieces of about 0.45 g were weighed to 10 μg accuracy, and put into 125-mL Pyrex bottles with 75 mL Milli-Q water, which were then sterilized by autoclaving for 60 min at 121°C. The water during autoclaving prevented the culm pieces from floating during the subsequent experiment, and extracted some DOC. This ensured that only microorganisms developing on submersed litter were studied. About 2 days after autoclaving, the water was replaced by 75 mL sterile artificial lake water and analyzed for DOC. All bottles were sealed with sterile gas-tight butyl rubber stoppers, either immediately to serve as sterile controls, or after inoculation according to Table 1, by adding 50 μL of "Fungi + bacteria" or "Bacteria" inoculum and/or a small piece (approximately 8 mm^3) from the actively growing edge of the mycelium of the precultured strain. Thereafter, the bottles were incubated at 10°C in darkness. Samples were taken from the inocula to assess the initial biomass upon inoculation.

Litter-Associated Bacteria

The detritus from each bottle was homogenized in 200 mL of Milli-Q water in a kitchen mixer during cooling on ice. Samples of 6.4 mL of homogenized slurry were analyzed for dry weight. Another 6.4 mL was preserved with 0.2-μm-filtered buffered formaldehyde (approximately 4% final concentration) and kept in 4°C, for analysis of litter-associated bacterial biomass. To detach bacteria from the litter, sodium pyrophosphate was added to a final concentration of 0.05 M, and the samples were incubated for 30 min [33]. Samples were sonicated using a probe (Microson XL Ultrasonic cell disruptor, Heat Systems or Rapids, Ultrasonics Ltd), 2 + 2 min with cooling on ice. The cells were then stained following a slightly modified version of a method by del Giorgio et al. [7]. A subsample of 0.5 mL was diluted with 2 mL of 0.2-μm-filtered water, stained with 7 μl SYTO 13 (Molecular Probes, Leiden, The Netherlands) for 15 min, and collected on 0.2-μm polycarbonate filters (Osmonics Inc.). After mounting in oil, the filters were stored frozen for at most 1 week until they were measured and counted in a Nikon epifluorescence microscope. At least 100 fields were analyzed, and between 30 and 315 cells (average 147) were counted and measured using an ocular micrometer. Sizing of bacteria by image-analysis of digital pictures was not feasible for litter-associated bacteria. The bacterial carbon content was calculated using the allometric conversion factor $CC = 218 \times V^{0.86}$, where CC is the cellular carbon content in femtograms and V is the bacterial volume in μm^3 [27].

Table 1. The dierent inocula added (x) to the dierent treatments in the main experiment

	Inoculum type		
Treatment	Bacteria	Fungi + bacteria	Cultivated fungi
Control	—	—	—
B	x	—	—
F + B	—	x	—
Cult F	—	—	x
Cult F + B	x	—	x

Biomass of Fungi

The remainder of the slurry was lyophilized and stored frozen until extraction and HPLC analysis of ergosterol, which was used as a measure of fungal biomass. Ergosterol was extracted using a slightly modified version of the method by Ek et al. [9], which is based on a protocol from Davis and Lamar [5]. All chemicals used had HPLC grade purity. About 150 mg of the dried litter was weighed to 10 μg accuracy and added to test tubes with 4 mL of 10% KOH in methanol and 1 mL of cyclohexane. The samples were sonicated for 15 min in an ultrasonic bath (Branson 2200) and after 45 min they were refluxed at 70°C for 90 min with caps tightly closed. After cooling to room temperature, 1 mL Milli-Q water and 2 mL cyclohexane were added and the samples were vortexed for 30 s. After centrifugation at 1000g for 5 min, the cyclohexane phase was transferred into new tubes and 2 mL of cyclohexane was added to the samples. After repeated centrifugation, the cyclohexane was transferred into the new tube, combining the two cyclohexane phases from the same sample. The cyclohexane was thereafter evaporated under a gentle stream of air at 40°C, and the samples were dissolved in 1 mL of methanol and incubated 15 min at 40°C. The samples were filtered through 0.45-μm PTFE syringe filters (Lida Manufacturing Corp., Kenosha, WI, USA) into autosampling vials and analyzed with a HPLC system (Gilson) equipped with a Phenomenex column (Sherisorb 5 ODS (2) 250 × 4.60 mm) and a UV detector set at 282 nm. Ergosterol standard (Fluka) was used to identify the peak, which appeared after about 12.5 min at room tem-

perature (approximately 23°C). The mobile phase was methanol at a flow rate of 1 mL min^{-1}. Fungal carbon biomass was estimated based on an ergosterol content of 5.5 mg g^{-1} fungal biomass [4, 15] and a 43% carbon content of fungi [1, 2].

Bacteria in the Water

Bacteria samples from the water (5 mL) were preserved with 4% final concentration of 0.2-μm-filtered buffered formaldehyde and kept at 4°C. The bacteria were counted in a flow cytometer (FACScan, Becton Dickinson) after staining with SYTO 13 (Molecular Probes, Leiden, The Netherlands) following a slightly modified protocol from del Giorgio et al. [7]. After centrifugation at 8200 *g* for 5 min, a working solution of the stain was prepared by diluting 20 times with 0.2-μm-filtered Milli-Q water. The samples were vortexed for at least 10 s before 1-mL subsamples were taken out and stained with 10 μL SYTO 13. An internal standard consisting of a known abundance of tracer particles (Fluoresbrite Carboxylate YG 1.5 Micron Microspheres, Polysciences Inc) was added to each sample. After at least 10 min staining, the samples were vortexed again and measured at low flow rate (12 μL min^{-1}). For the calculations of carbon turnover, biomass determination of water bacteria by image analysis was conducted. All samples from day 79 were collected on filters according to the protocol for litter-associated bacteria except for the steps prior to staining. Before subsampling, the vials were vortexed for 1 min, and 2 μL SYTO 13 was used for staining. The cells were photographed using a Nikon Eclipse E600 microscope equipped with a Nikon DXM1200 digital camera. At least 30 cells from each sample (15 cells in control and Cult F samples) were measured using the software Easy Image Analysis 2000 (Bergström Instrument AB, Solna, Sweden). The volume of each cell was calculated approximating each cell as a cylinder with hemispherical ends. Cell carbon content was derived from cell volume as described for litter-associated bacteria.

Carbon Dioxide

Samples for carbon dioxide analysis were withdrawn from both gas phase and water of each bottle, using a syringe to penetrate the rubber stoppers. From the headspace, either the gas samples were immediately analyzed, or 6 mL was stored dark at 10°C for a maximum of 2 months in nitrogen-rinsed evacuated 15-mL Vacutainers (Hemogard Z, Becton Dickinson Vacutainer Systems Europe, Meylan Cedex, France). Water samples were analyzed using the headspace equilibration method [18]. Helium (10 mL) was added to 10 mL sample water in a plastic syringe equipped with a valve. In order to transfer all inorganic carbon species into CO_2, 100 μL 1.2 M HCl was added. The syringe was shaken vigorously for 1 min to reach equilibrium between the water and gas phase. The headspace gas was extracted and either analyzed immediately or stored as described above for the gas-phase samples. All samples were analyzed with a gas chromatograph (GC-14A, Shimadzu) equipped with a Poropak Q column and a thermal conductivity detector. Subsamples of 0.8 mL were injected into the chromatograph, using helium as a carrier gas with a flow of 40 mL min^{-1}. A certified gas mixture with 1616 ppm CO_2 (AGA Gas AB, Sweden) was used for calibration. The content of carbon dioxide in each bottle was calculated as the sum of CO_2 in gas phase and water.

Dissolved Oxygen

To ensure that the levels of dissolved oxygen were adequate, the concentrations were measured in the water of bottles retrieved at days 17, 49, 65, 79, and 93. Samples of 5 mL were withdrawn with a syringe through the rubber stopper and replaced by 5 mL sterilized artificial lake water in order to prevent underpressure. Oxygen was analyzed with a spectrophotometric Winkler method, modified after Roland et al. [28]. Mn^{2+} solution (25 μL, 3.0 M Mn^{2+}, 6.0 M Cl^-) was added along with 40 μL of $OH^-I^-N_3^-$ (8.0 M OH^-, 4.0 M I^-, 0.55 M N_3^-, 12 M Na^+) solution to the syringe, which was then sealed with Parafilm M. After thorough shaking, 50 μL of concentrated H_3PO_4 was added and the syringe was shaken until the precipitation was dissolved. The concentration of dissolved O_2 (mg $liter^{-1}$) was derived from the absorbance at 450 nm, using the equation below, which was derived from calibration with Winkler titration.

$$[\text{dissolved oxygen}] = 11.915 \times A_{450,1\text{cm}} - 0.097568$$

Dissolved Organic Carbon

DOC samples were stored in darkness in 15-mL Falcon tubes (Becton Dickinson, Meylan Cedex, France) at 4°C for 4–8 weeks. According to previous experience, the DOC concentration of this type of sample is not affected by this storage procedure. The samples were analyzed as nonpurgable organic carbon by high-temperature combustion on a Shimadzu TOC-5000 carbon analyzer.

pH

The pH was measured during stirring on a Crison micropH 2001 (Crison Instruments, S.A. Barcelona, Spain), which was corrected for the temperature in the samples.

Carbon and Nitrogen in the Litter

For analysis of carbon and nitrogen content and dry weight of remaining litter, 6.4 mL of the homogenized slurry was collected in pre-weighed glass vials, dried overnight at 95°C, and stored in a dessicator until analysis. Approximately 2-mg samples of detritus were weighed on a CAHN/Ventron 4700 balance (Thermo Cahn, Madison, WI, USA), placed in tin capsules, and analyzed for particulate carbon and nitrogen on a Leco CHNS-932 analyzer.

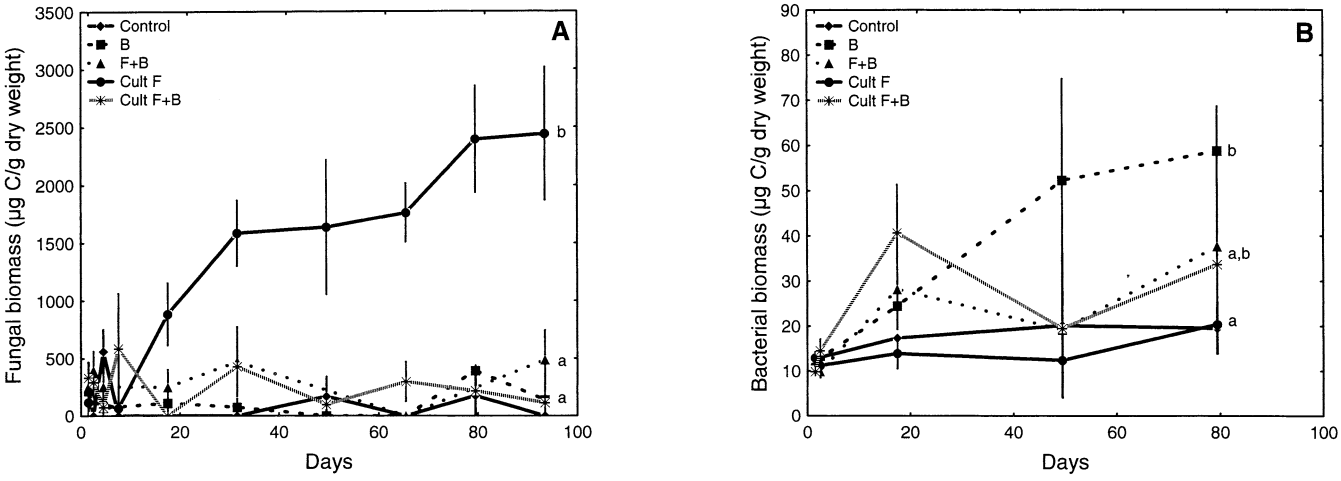

Fig. 1. Biomass of (A) fungi in the different treatments and (B) litter-associated bacteria in the main experiment. Bars indicate ± 1 SE. In some cases, error bars are hidden within symbols. Treatments with different letters were statistically different ($p < 0.05$).

Statistical Analyses

All measured variables except pH and carbon and nitrogen content were log-transformed in order to stabilize the variance. The residuals were normally distributed after transformation [30]. A two-way ANOVA of each measured variable was performed, with days from experiment start and treatment (type of microbial inoculum) as factors. For the bacteria in the water, a one-way ANOVA was performed on the data from day 79. Where not stated otherwise, differences were considered significant at $p < 0.05$. In order to find differences in treatment effect between specific treatments, post hoc comparisons (Tukey's HSD test) were used. Treatments effects on the carbon turnover were analyzed by one-way ANOVA of each variable. The computations were performed using STATISTICA 6.0 (StatSoft, Inc.).

Results

Fungal biomass development differed between the treatments, with highest fungal growth in the absence of bacteria (Cult F bottles) from day 17 and throughout the experiment. Cult F was substantially different from all other treatments (Fig. 1A, Table 2). Litter-associated bacterial biomass (Fig. 1B) also differed between treatments, with highest final biomass where bacteria grew in absence of fungi (treatment B). Due to the considerable variation among replicates only the differences between B and the control and Cult F were significant (Fig. 1B). In the comparison of five different fungal strains, fungi accumulated substantially more biomass when inoculated alone than together with bacteria ($p < 0.001$, Fig. 2) and there were no significant differences between the different fungal strains ($p = 0.73$), or interaction between strain and bacterial presence ($p = 0.51$; two-way ANOVA).

In the absence of bacteria, a net accumulation of fungal biomass corresponding to 2400 µg C g^{-1} dry weight took place, but when bacteria were present only about 200 µg C g^{-1} accumulated. The bacterial biomass accumulated at

Table 2. Results from the two-way ANOVA, where the eects of treatment, time and the interaction between treatment and time were tested for each measured variable (ns, nonsignificant)

	p-values for dierent sources of variance		
Variable	Treatment	Time	Treatment × time
Fungal biomass	<0.001	ns	ns
Litter-associated bacterial biomass	<0.01	<0.001	ns
Dissolved oxygen concentration	<0.001	<0.001	ns
Carbon dioxide concentration	<0.001	<0.001	<0.001
pH	<0.001	<0.001	ns
Carbon content (% of detritus)	ns	<0.001	ns
Nitrogen content (% of detritus)	ns	ns	ns
Dissolved organic carbon	<0.01	<0.001	<0.001
Bacterial biomass in water	<0.001	—	—

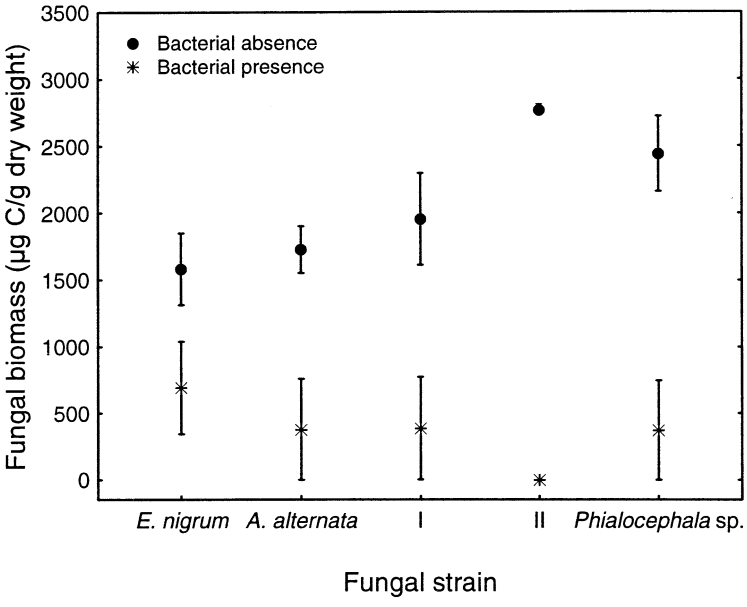

Fig. 2. The biomasses of the different fungal strains at the end of the complementary study (after 50 days) were significantly different in presence and absence of bacteria ($p < 0.001$). Bars indicate ± 1 SE. In some cases, error bars are hidden within symbols.

the end of the experiment was around 60 µg C g^{-1} dry weight in absence of fungi, but only 33 µg C g^{-1} in fungal presence. Bacterial biomass in the water differed between the treatments (Table 2). The highest biomass was accumulated in treatment B, which was significantly different from all other treatments except for Cult F + B.

Dissolved oxygen concentrations decreased throughout the experiment in all inoculated bottles, at approximately the same rate. In the control bottles, the oxygen concentration remained at a high and stable level throughout the experiment, demonstrating that there was not any notable microbial activity in the controls (Fig. 3A). The carbon dioxide increased in all inoculated bottles and Tukey's HSD test showed that the control and Cult F + B were different from all other treatments (Fig. 3B). The pH decreased slowly in all bottles during incubation, but to a larger extent in the inoculated ones than in control bottles (Fig. 3C). Also, the inoculated bottle with highest pH (F + B) was significantly different from the one with lowest (Cult F + B). There were no differences in carbon or nitrogen content in the litter between any of the treatments (Table 2). However, the relative carbon content increased in all treatments during the experiment. The DOC values were at most times highest in the control bottles, but there was a large variation over time in most treatments (Fig. 3D).

The calculations of carbon turnover made on data from sampling day 79 showed that the absolute majority of the carbon remained as detrital matter (Table 3). About 2–3% of the carbon was respired in the inoculated bottles, with highest respiration rates in treatments B and Cult F + B. In general, microbial biomasses constituted a minor proportion of the carbon (Table 3). The higher fungal growth in treatment Cult F resulted in fungi representing a considerably larger fraction of the total carbon than in other treatments. The proportion of DOC was highest in the control and lowest when fungi and bacteria co-occurred (Cult F + B). Overall, the interactions of fungi and bacteria resulted in changes in the relative contribution of each group of degraders to total microbial biomass (Fig. 1), as well as in their contribution to total carbon content of the litter system (Table 3). However, ecosystem functioning at the level of overall carbon metabolism was at most marginally dependent on whether bacteria and/or fungi were present in the degrader community (Fig. 3).

Discussion

Both fungi and bacteria grew better in the absence of one another than when they occurred together. This result suggests a bilateral antagonistic relationship between these two major groups of heterotrophic microorganisms. Albeit the bacterial biomass was generally much lower than the biomass of fungi, the presence of bacteria clearly obstructed the development of a large fungal biomass. This highly significant effect could be detected within 2 weeks after inoculation and has not been shown previously. The similar result with five different fungal strains (Fig. 2) suggests that the antagonism is a generally occurring

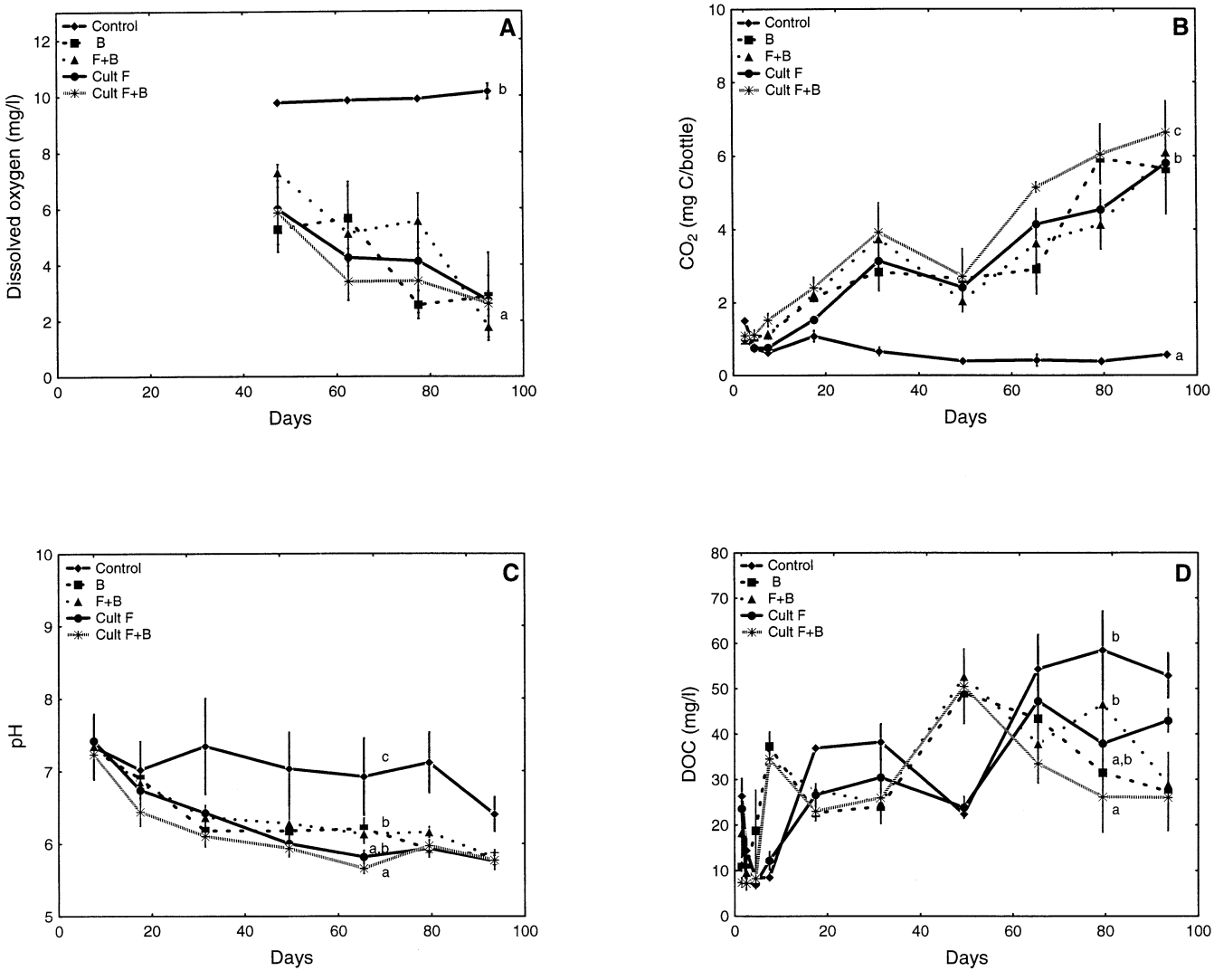

Fig. 3. Temporal development in the different treatments in (A) concentrations of dissolved oxygen from day 49 to day 93, (B) concentrations of inorganic carbon derived from carbon dioxide from day2 to day 93, (C) pH from day 7 to day 93 and (D) concentrations of dissolved organic carbon throughout the experiment. Bars indicate ± 1 SE. In some cases, error bars are hidden within symbols.Treatments with different letters were statistically different ($p < 0.05$).

phenomenon. We suggest that this antagonism between the groups may be an important controlling factor for colonization and decomposition of plant material in aquatic environments. The same controlling effect from bacteria on fungi appeared in both inoculation types with fungi and bacteria grown together (F + B and Cult F + B) and the result was supported by the complementary study, showing the same effect for all fungal strains tested.

In a study similar to ours, Wohl and McArthur [35] investigated actinomycete–fungal interactions in aquatic microcosms and found a similar antagonistic effect on fungi from the bacteria. Fungi did not grow better if inoculated 2 days prior to bacteria than at simultaneous inoculation. The cause for inhibited fungal growth was not chitinolysis by bacteria as hypothesized, but rather presence of a bacterial extracellular compound, competition for colonization substrata, or direct competition for trace elements or nutrients. Competition for POM (particulate organic carbon) was not considered probable, since bacteria utilized primarily DOC, while fungal nutrition depended to a larger extent on POM [35]. However, in that experiment, levels of DOC were probably substantially higher than in the present study, since the water in the bottles was not changed after autoclaving. De Boer et al. [6] showed antifungal properties in chitinolytic soil bacteria and suggested that this was due to a combination of antibiotics and lytic enzymes. Again, simple competition for resources was excluded as an explanation, since the

Table 3. The distribution of carbon at day 79 expressed as percentages of the initial carbon content in the bottles ± standard errors[a]

Variable	Detrital matter	Dissolvec organic carbon	Inorganic carbon (i.e. C in medium and CO_2)	Fungal biomass	Litter-associated bacterial biomass	Water bacterial biomass
p-value	<0.01	ns	<0.001	<0.001	<0.05	<0.001
Control	97.6 ± 0.3	2.13 ± 0.31	0.19 ± 0.03	0.04 ± 0.04	0.004 ± 0.001	0.025 ± 0.002
B	95.6 ± 0.2	1.16 ± 0.10	2.94 ± 0.20	0.09 ± 0.01	0.013 ± 0.002	0.153 ± 0.028
F + B	96.3 ± 0.3	1.67 ± 0.14	1.99 ± 0.34	0.01 ± 0.01	0.008 ± 0.002	0.059 ± 0.019
Cult F	95.8 ± 0.1	1.42 ± 0.32	2.21 ± 0.21	0.53 ± 0.10	0.005 ± 0.001	0.001 ± 0.001
Cult F + B	96.0 ± 0.2	0.95 ± 0.30	2.90 ± 0.39	0.05 ± 0.03	0.007 ± 0.001	0.119 ± 0.022

[a] The treatment effects from the one-way ANOVA are given as *p*-values for each variable. ns, nonsignificant.

medium used contained the necessary nutrients [6]. In the present study, resource competition could be approved as a reason for the antagonism. However, bacterial growth continued for an extensive period of time after they were first detected to inhibit fungi (Figs. 1A and 1B). Hence, although there may have been competition for resources, the suppression of fungi was not likely to be due only to bacterial exhaustion of resources.

The fraction of detritus being degraded was small compared to some previous field observations [e.g., 1]. We examined the response of carbon turnover in experimental microcosms designed to study fungal–bacterial interactions, and the carbon turnover results cannot easily be extrapolated to natural conditions. Possible explanations for the slow degradation include low temperature and low concentrations of dissolved oxygen, since oxygen levels decreased in all inoculated bottles throughout the experiment. Neither of these factors should, however, cause highly retarded metabolism, since decomposition of macrophytes is known to occur also during late fall and winter [13, 20], when both oxygen concentrations and temperature tend to be low. The culms of *Phragmites australis* are very recalcitrant, remaining in lakes for a considerable period after death [17]. This is probably the main reason for the slow degradation of the culms, which have also been shown to decompose slowly in nature [13, 19, 20]. Moreover, the culms were partly degraded already at the beginning of the experiment, resulting in lack of the most labile fraction of the overall recalcitrant matter and dominance of lignocellulosic tissue. Hence, our study of bacterial–fungal interactions was conducted with a substrate that was probably very recalcitrant, with a minimal contribution of labile substrates moieties.

The largest biomasses of bacteria and fungi in the experiment were low compared to values reported from field investigations [1, 8, 14, 20]. However, the relative contribution of fungi and bacteria to total biomass in microcosms where both groups were present was similar to typical field observations, i.e., dominance by fungi, constituting ≥90% of the microbial biomass [1, 11, 20, 21]. Also, the other variables monitored during the experiment concur with field measurements. Hence, oxygen and temperature as well as pH were within the range of typical values.

Despite the dramatic influence on development of bacteria and fungi, the different treatments had no substantial effects on the carbon metabolism in the experiment. Except for the control bottles, there were rather small differences in remaining litter dry weight, carbon dioxide development, and oxygen consumption. The substantial biomass of fungi developing when bacteria were absent (treatment Cult F) was not higher in respiration than any of the other inoculated treatments, a result supported by the similar levels of dissolved oxygen.

There was a background level of ergosterol in some bottles from each treatment, causing a large variance in fungal biomass in all treatments where the development of fungal biomass was low (Fig. 1A). The background level of ergosterol found in those bottles is in the low range of what can be found on decaying litter in nature, or lower [14, 16, 22, 24]. Still, it was substantially higher than the detection level of the method and it could be measured with a precision similar to the precision of measurements of the higher levels in the Cult F treatment. This background level also remained without decreasing throughout the experiment in bottles that contained bacteria. This strongly suggests that the bacteria did not degrade ergosterol. Thus, the lack of increase in ergosterol content in presence of bacteria was reflecting the lack of development of a fungal community, rather than bacterial degradation of the ergosterol.

Also for litter-associated bacterial biomass, there was a background level, since the control and the Cult F treatments had measurable bacterial biomass. This was prob-

ably due to autoclaved bacteria present on the detritus before experiment startup. The constant levels of dissolved oxygen, pH, and carbon dioxide indicate that the control bottles remained sterile.

In conclusion, we demonstrate a strong antagonistic effect from bacteria on fungi, since the bacterial presence effectively suppressed fungal growth despite of otherwise suitable fungal growth conditions. We also observed a reverse effect, from fungi on bacteria, and suggest this bilateral antagonism to be an important controlling factor for colonization and growth of microbial degraders.

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