Ecology of mycophagous collimonas bacteria in soil

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MYCOPHAGOUS GROWTH OF *COLLIMONAS* BACTERIA IN NATURAL SOILS, IMPACT ON FUNGAL BIOMASS TURN-OVER AND INTERACTIONS WITH MYCOPHAGOUS *TRICHODERMA* FUNGI

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Abstract

Bacteria of the genus *Collimonas* are widely distributed in soils albeit at low densities. In the laboratory, they were shown to be mycophagous i.e. they are able to grow at the expense of living hyphae. However, so far the importance of mycophagy for growth and survival of collimonads in natural soil habitats is unknown. Using a *Collimonas*-specific real-time PCR assay, we show here that invasion of field soils by fungal hyphae (*Absidia* sp.) resulted in a short-term, significant increase (average 4-fold) of indigenous collimonads. No such responses were observed for other soil bacteria studied (*Pseudomonas, Burkholderia,* PCR-DGGE patterns of total bacteria and *Burkholderia*). Hence, it appears that the stimulation of growth of *Collimonas* bacteria by fungal hyphae is not common among other soil bacteria. In the same field soils, *Trichoderma*, a fungal genus known for mycophagous (mycoparasitic) growth, increased upon the introduction of *Absidia* hyphae. Hence, mycophagous growth by *Collimonas* and *Trichoderma* can occur in the same soils. However, in controlled experiments (sand microcosms) collimonads appeared to have a negative effect on mycophagous growth of a *Trichoderma* strain. The effect of mycophagous growth of collimonads on fungal biomass dynamics was studied in sand microcosms using the same *Absidia* sp. as a test fungus. The growth of collimonads did not cause a significant reduction of the *Absidia* biomass.

Overall the study indicates that mycophagous nutrition may be important for collimonads in natural soils, but the impact on fungal biomass turn-over is likely to be minor.

INTRODUCTION

Mycophagy, i.e. the feeding on living fungi, has been reported for soil bacteria of the genus *Collimonas* [10, 17]. Mycophagous growth was based on the proliferation of collimonads in gnotobiotic sand microcosms that contained living fungal mycelium as the only source of energy and carbon [7]. However, whereas the nutrient poor conditions in these microcosms were realistic for natural soils, other conditions, e.g. the absence of other (micro) organisms and plant roots, were different from the natural soil environment.

In a field inventory we investigated the distribution of collimonads among different soils [63]. Collimonads appeared to be more abundant in fungal-rich natural grassland and forest soils than in fungal-poor arable soils. Yet, no clear relationship between fungal biomass and abundance of collimonads was observed. Hence, further studies are required to elucidate the importance of mycophagy for *in situ* growth of collimonads, in particular because they can grow on a wide range of organic substrates, i.e. they are facultative mycophagous [15].

Unlike the few studies that have been carried out on bacterial mycophagy, fungal mycophagy, which is better known as mycoparasitism, has been the subject of many studies [5, 27]. Most of these studies deal with *Trichoderma* species [29, 69]. In particular, the application of *Trichoderma* spp. to control plantpathogenic fungi (e.g. *Rhizoctonia spp.* and *, Fusarium spp*) has received much attention [5, 70, 71]. *Trichoderma* spp. are widely distributed in the terrestrial environment [72]. However, as for collimonads, the actual importance of mycophagous growth for *Trichoderma* spp. under natural soil conditions is not known. Like collimonads, *Trichoderma* spp. are facultative mycophagous [15]. Hence for both collimonads and *Trichoderma* spp. other sources of energy, e.g. soil organic matter and root exudates, may be more important for their growth than fungal-derived substrates.

The current study was aimed (1) to find evidence for the importance of mycophagous growth for collimonads in natural soils, (2) to assess the consequence of mycophagous growth of collimonads for fungal biomass

production and (3) to examine possible interactions between collimonads and *Trichoderma* spp. to perform their mycophagous growth.

MATERIAL AND METHODS

Soils. In January 2007, soil samples (upper 10 cm of mineral layer) were collected from sites, where collimonads had been detected earlier [63]. The soils were a forest soil (site 2), 2 grassland soils (site 4 and 5) and a soil from an abandoned arable site (site 22), respectively. Characteristics of these soils (site 2, 4, 5 and 22) were described previously as pH_{water} 3.5, 4.9, 5.3 and 5.6, total organic carbon 36.1, 9.6, 77.3 and 31.1 g kg^{-1} and C/N ratio 26.8, 11.9, 10.2 and 19.2, respectively [63].

Bacterial strains. The strains used in this study were *Collimonas fungivorans* Ter 331 (AJ310395) and Ter 6^T (LMG 21973), *C. arenae* Ter 10^T (LMG 23964) and *C. pratensis* Ter 91^T (LMG 23965), respectively [17, 73]. These 4 strains have been isolated from the same soil [17]. Hence, their co-occurrence is natural. Based on the experimental conditions and feeding-preferences, the mixture of the 4 strains was used to provide the best chances for mycophagous growth in this study (De Boer *et al*., 2004). *Pseudomonas fluorescens* strain AD21 is a soil isolate that has been described before [74]. *Burkholderia* JS is a soil isolate kindly provided by Dr. Drigo (Plant Research International, Wageningen, The Netherlands) which has 98% identity with a *Burkholderia cepacia* strain (AY741358).

Fungal strains. Based on earlier work, it was evident that growth of collimonads can be especially stimulated by zygomycetal fungi [7]. In the current study we used a zygomycetal fungus, isolated from a grassland soil, that was identified as *Absidia* sp. on basis of the sequence analysis of the internal transcribed spacer (ITS) region in the nuclear ribosomal repeat unit, using primers ITS1-F and ITS4-B [75]. *Absidia* spp. are common saprotrophic soil fungi [76]. *Trichoderma harzianum* CECT 2413 [77] was purchased from the Spanish type culture collection - CECT (University of Valencia, Spain).

Experiment 1: Growth responses of indigenous* Collimonas *spp.,* Pseudomonas *spp.,* Burkholderia *spp. and* Trichoderma *spp. to invasion of field soils by* Absidia *mycelium Soils collected from each sampling site were homogenized and portions (40g) of the soils were transferred to Petri dishes (diameter, 8.5 cm) and spread evenly. A potato dextrose agar (PDA) disk (diameter, 1 cm) from the growing margin of *Absidia* was inverted and placed on an autoclaved metal slide and was centrally placed in the middle of the Petri dish. The metal coin was used to prevent leaching of nutrients from the agar disks into the soil. The Petri dishes were sealed and incubated at 20 °C. After 1 week of incubation, *Absidia* mycelium covered the whole soil surface. Samples were taken by scraping the soil surface after 0, 2 and 3 weeks of incubation. In addition, comparable samples were taken from Petri dishes without invading *Absidia* hyphae. For all soils, treatments (with and without *Absidia*) were done in 6-fold.

Soil DNA extracted from the collected soil samples was used for real-time PCR enumerations of collimonads and fungi belonging to the genus *Trichoderma*. Real-time PCR enumerations of indigenous *Pseudomonas* and *Burkholderia* bacteria as well as PCR-denaturating gradient gel electrophoresis (PCR-DGGE) analysis of the bacterial community structure were performed to evaluate the specificity of the growth increase of collimonads upon introduction of fungal mycelium. DNA was extracted from an amount of soil equivalent to 0.25 g dry soil using the MOBIO kit (MOBIO laboratories, Solana Beach, CA) according to the manufacturer's instruction, except that soil DNA was finally eluded in 50 µl instead of 100 µl.

Experiment 2: Estimation of the mycophagous biomass production by collimonads and evaluation of interactions between collimonads and* T. harzianum *upon mycophagous performance in gnotobiotic sand microcosms. Gnotobiotic sand microcosms were used to study the impact of *Collimonas* spp. on fungal biomass production. *Collimonas* strains were pre-grown on chitin-yeast agar

at 20°C for 14 days as described by de Boer *et al*. [7]. *Absidia* sp. was grown on potato-dextrose agar (PDA) at 20 °C for 4 days.

The 4 different *Collimonas* strains were mixed by adding equal numbers of cells of each strain to P-buffer (KH_2PO_4, 1 g L^{-1} [pH 6.5]). The suspension was mixed into autoclaved, acid-purified beach sand to give a moisture content of 5 % (wt/wt) and a total bacterial density of 10^4 cells g^{-1} of sand (based on microscopic counts), which is a common density of collimonads in field soils [63]. Portions (40 g) of the incubated sand were transferred to Petri dishes (diameter, 8.5 cm) and spread evenly. The Petri dishes were sealed, placed at 20 °C, and pre-incubated for 1 week to allow the micro-organisms to adapt to the prevailing conditions. Next, an agar disk (PDA; diameter, 1 cm) from the growing margins of *Absidia* was inverted and placed on an autoclaved metal slide and this was centrally placed in the middle of Petri dish. The Petri dishes were sealed and incubated at 20 °C. After 3 weeks of incubation, sand was removed from the surface covered by hyphae of *Absidia* and homogenized before measurement of fungal biomass (ergosterol) and abundance of collimonads (real-time PCR).

The same experimental set-up was used to study interactions of collimonads with the mycophagous fungus *Trichoderma harzianum* during growth on *Absidia* hyphae.

Sterile sand with or without collimonads (see above) was inoculated with a spore suspension (10^4 spores g^{-1} sand) of *T. harzianum*. Conidia had been produced on PDA and were collected and suspended in P-buffer. After vortexing, the suspension was centrifuged at 4000 rpm for 1 minute. The supernatant was collected as spore suspension and was mixed into autoclaved, acid-purified beach sand to give a moisture content of 5 % (wt/wt) and a total density of 10^4 spores g^{-1} of sand (based on microscopic counts). All treatments were done in 6-fold. Using the prepared microcosms in the same experimental set-up with the inoculation of *Absidia* (see above), Petri dishes were sealed and incubated at 20 °C. After 3 weeks of incubation, sand was removed from the surface covered by hyphae of

Absidia and homogenized before measurement of abundance of collimonads and *T. harzianum* (real-time PCR).

Real-time PCR. Real-time PCR was performed on a Rotor-Gene 3000 (Corbett Research, Sydney, Australia). All mixes were made using a CAS-1200 pipetting robot (Corbett Research, Sydney, Australia) to reduce variation caused by pipetting errors. Quantification of collimonads in soil DNA samples was performed as described by Höppener-Ogawa *et al.* [78].

For quantification of pseudomonads, *Burkholderia* spp. and *Trichoderma* spp., the Absolute qPCR SYBRGreen mixture (ABgene) was used at a final concentration of 1× for the real-time reaction. The standard curves for *Pseudomonas* and *Burkholderia* were made from genomic DNA extracted from a pure culture of *Pseudomonas fluorescens* strain AD21 and *Burkholderia* JS (see strain description). The *Pseudomonas* specific primer sets used were PSf (5'-GGT CTG AGA GGA TGA TCA GT-3') and PSr (5'-TTA GCT CCA CCT CGC GGC-3')[79]. The *Burkholderia* specific primer sets used were Burk3 (5'- CTG CGA AAG CCG GAT -3') and BurkR (5'- TGC CAT ACT CTA GCY YGC -3')[80].

For quantification of *Trichoderma* spp., we used a modified protocol of Hagn *et al.* [78]. The *Trichoderma* standard curve was made from DNA extracted from a pure culture of *T. harzianum* CECT 2413. PCR-amplification was done using the fungal universal primer sets NS1 (5'-GTA GTC ATA TGC TTG TCT C-3') [81] and TW13 (5'-GGT CCG TGT TTC AAG ACG-3')[82].

For quantification of *Absidia* sp.*,* real-time PCR quantification was done using universal fungal specific primers as described elsewhere [83]. In addition, measurement of the fungal cell membrane component ergosterol via an alkaline extraction protocol was performed to quantify the biomass of *Absidia* sp. [47].

PCR-denaturing gradient gel electrophoresis analysis. All PCR reactions and denaturing gradient gel electrophoresis (DGGE) were carried out as described elsewhere [80, 84]. The primers 968f-GC, 1378r [85] and FR1-GC, FF390r [83]

were used to analyze bacterial and fungal communities, respectively. The nested PCR reactions were performed for *Burkholderia* spp. with primer Burk3, R1378 [80]. The products from the first PCR were diluted 1:1,000 and used as the template in the second PCR with primers Burk3-GC and BurkR [80].

Statistical analyses. The banding patterns of DGGE gels were analyzed using the Image Master 1D program (Amersham Bioscience, Roosendaal, the Netherlands). The resulting binary matrices were exported and used in statistical analysis as "species" presence-absence matrices.

The effect of the introduction of *Absidia* hyphae, sampling site and the interaction of these two factors on the community structure as analyzed by PCR-DGGE was tested by distance-based redundancy analysis [86]. Jaccard's coefficient of similarity was calculated and the resulting similarity matrix was exported to Canoco 4.5 as species data for redundancy analysis (RDA) [51]. Variables to be included in the model were chosen by forward selection at a 0.05 baseline. The significance of canonical model was tested with 999 permutations. To test the effects of each of the two variables (*Absidia* invasion and soil origin), the individual variables were recorded using dummy binary-variables of which one was used in Canoco as the only environmental variable in the model and the other as co-variable. To test the interaction, the only variable entered in the model was the interaction between *Absidia* invasion and soil origin, while both individual factors were included (without interaction) as co-variables. The significances of such models were tested with 999 permutations.

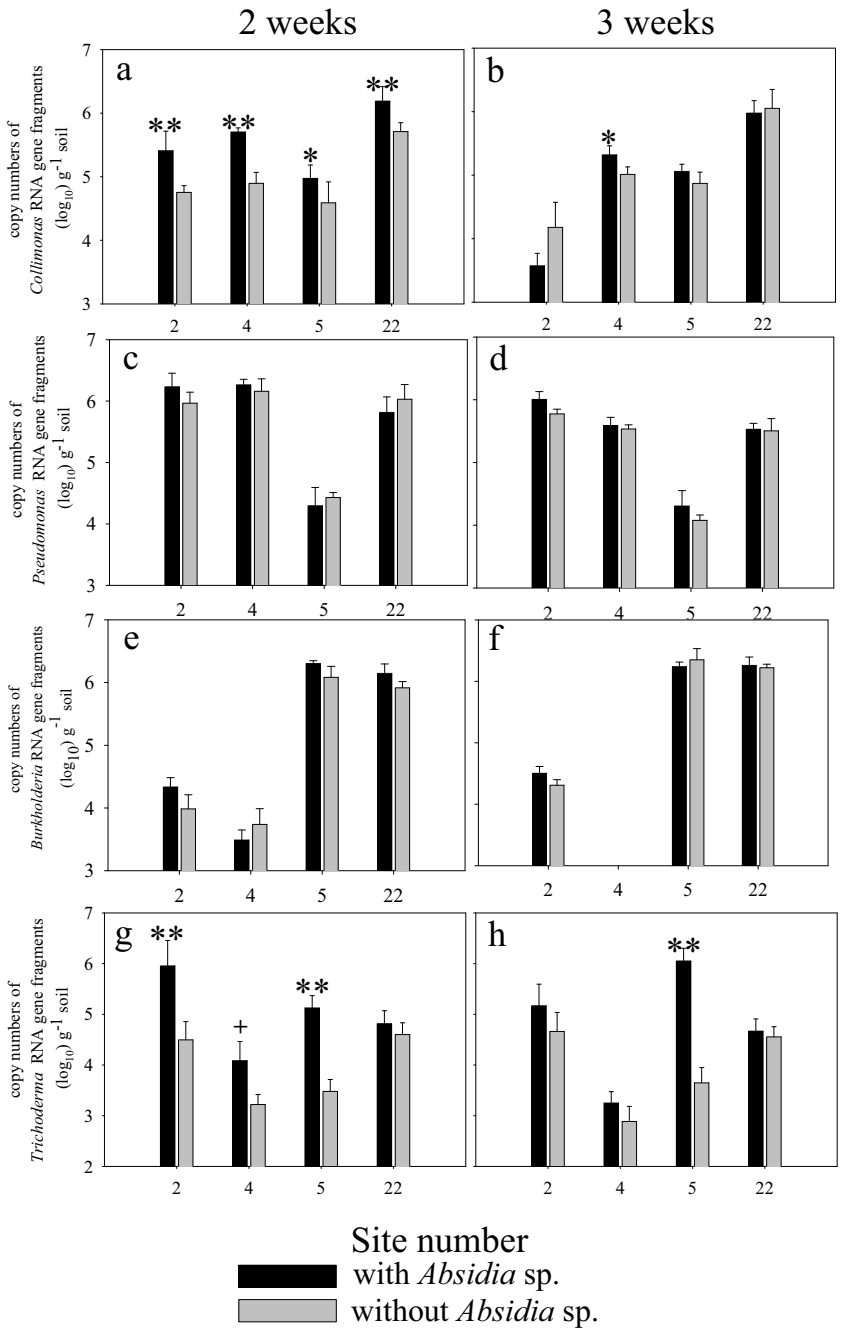
All ANOVAS were performed using Statistica 7.0 (StatSoft Inc., Tulsa, OK). For ANOVA, normal distribution of data was tested with Shapiro-Wilks test and variance homogeneity by Levene's test. When data failed to satisfy one of the tests, an appropriate transformation was applied (log or square root transformation). Tukey's honest significant difference (HSD) method modified for unequal sample size (Unequal N HSD in Statistica) $P < 0.05$ was used.

RESULTS

Growth response of indigenous collimonads and* Trichoderma *spp. to invasion of field soils by* Absidia *mycelium (experiment 1). The effect of introduction of *Absidia* mycelium on the numbers of collimonads is given in FIG 4.1a and 4.1b. The real-time PCR-based numbers of indigenous collimonads in the soils were 5.0 $\times 10^4$ g^{-1} soil for site 2, 4 and 5 and 7.5 $\times 10^5$ g^{-1} soil for site 22. These numbers did not increase during 2 weeks incubation without introduction of fungal hyphae. Numbers of collimonads had increased (4.1 fold on average) in all 4 soils, 2 weeks after invasion by *Absidia* hyphae. However, with the exception of soil 4, the stimulating effect of invasion by *Absidia* hyphae was no longer apparent when the incubation period was one week longer (3 weeks in total).

The growth dynamics of two bacterial genera, *Pseudomonas* and *Burkholderia,* were examined to have an indication of the specificity of the growth response of collimonads upon introduction of *Absidia.* No effects of introduction of *Absidia* on bacterial numbers within these genera were detected (FIG 4.1c to 4.1f). The numbers of *Burkholderia* bacteria in soil 4 were under detection limit (1.0×10^3 copies g^{-1} soil) after 3 weeks incubation.

FIG 4.1: Growth response of indigenous soil bacteria *belonging to the genus Collimonas (1A,1B), Pseudomonas (1C,1D) , Burkholderia (1E,1F) and of indigenous soil fungi belonging to the genus Trichoderma (1G,1H) upon extension of mycelium of the fungus Absidia in 4 field soil microcosms (site 2, 5, 4 and 22). Copy numbers of the 16S rRNA of the bacterial genera and of the ITS region of Trichoderma rRNA were determined by real-time PCR in the mycelial zone of Absidia (black bar) and in a comparable zone of microcosms without introduction of Absidia (gray bar). Real-time PCR based quantifications were done 2 and 3 weeks after introduction of the fungal inoculum. Data represent the means and standard deviations for six replicates that were harvested at the indicated times. Different symbols within a graph indicate significant differences for a particular soil between microscosms with and without introduction of Absidia (+ ; $P \leq 0.1$, * ; $P \leq 0.05$, ** ; $P \leq 0.01$) based on Tukey's HSD test.*


2 weeks
3 weeks
a
b
c
d
e
f
g
h
copy numbers of Collimonas RNA gene fragments (log10) g-1 soil
copy numbers of Pseudomonas RNA gene fragments (log10) g-1 soil
copy numbers of Burkholderia RNA gene fragments (log10) g-1 soil
copy numbers of Trichoderma RNA gene fragments (log10) g-1 soil
2
4
5
22
Site number
with Absidia sp.
without Absidia sp.

Real-time PCR-based numbers of indigenous fungi belonging to the genus *Trichoderma* were significantly increased in 2 soils (soil 2 and 5), two weeks after introduction of *Absidia* (FIG 4.1g and 4.1h). In soil 4, the increase was significant at the level of $P < 0.1$. The average increase in real-time PCR based numbers in these 3 soils was 26.8 fold. After 3 weeks of incubation, a significant stimulation of abundance of *Trichoderma* spp. was only found for soil 5.

Impact of soil colonization by* Absidia *mycelium on bacterial and fungal community structure (experiment 1). The effect of invading *Absidia* mycelium on microbial community structure as assessed by the PCR-DGGE in the different soils was analyzed by db-RDA (Table 4.1)(Appendix FIG 4.4). The bacterial community structure, including *Burkholderia* community structure, appeared to be unchanged whereas the fungal community structure was significantly influenced by introduction of the *Absidia* in all the soils that we tested. For all soils, the band corresponding to *Absidia* was clearly visible in the gel.

Table 4.1: Distance-based redundancy analyses (db-RDA) *of the effect of invasion of hyphae of the fungus Absidia sp. on microbial community structures (presence-absence of PCR-DGGE bands) in microcosms of 4 different field soils.*

Incubation time		Bacteria		Fungi	
	Factors	**F-ratio**	**P-value**	**F-ratio**	**P-value**
2 weeks	*Absidia*	0.900	0.518	3.069	*0.001*
	Soil origin	42.907	*0.001*	3.088	*0.001*
	Absidia × Soil	0.900	0.464	2.629	*0.001*
3 weeks	*Absidia*	0.153	0.967	3.399	*0.007*
	Soil origin	21.666	*0.001*	4.399	*0.001*
	Absidia × Soil	0.071	1.000	2.728	*0.001*

Growth response of* Absidia *to* Collimonas *mycophagy (experiment 2). The introduction of *Absidia* in purified sand containing collimonads resulted in a 5.2 fold increase of the real-time PCR-based number of collimonads in the mycelial zone (FIG 4.2a). The dynamics of the abundance of *Absidia* was assessed by quantification of 18S rRNA gene copies and ergosterol (FIG 4.2b and 4.2c, respectively). Results of real-time PCR indicated that 18S rRNA gene copies of the *Absidia* tended to increase in the presence of collimonads although the differences

between controls and collimonads-containing microscosms were not significant at the 5% level. The quantities of the fungal membrane component ergosterol did not differ significantly between controls and collimonads-containing microscosms.

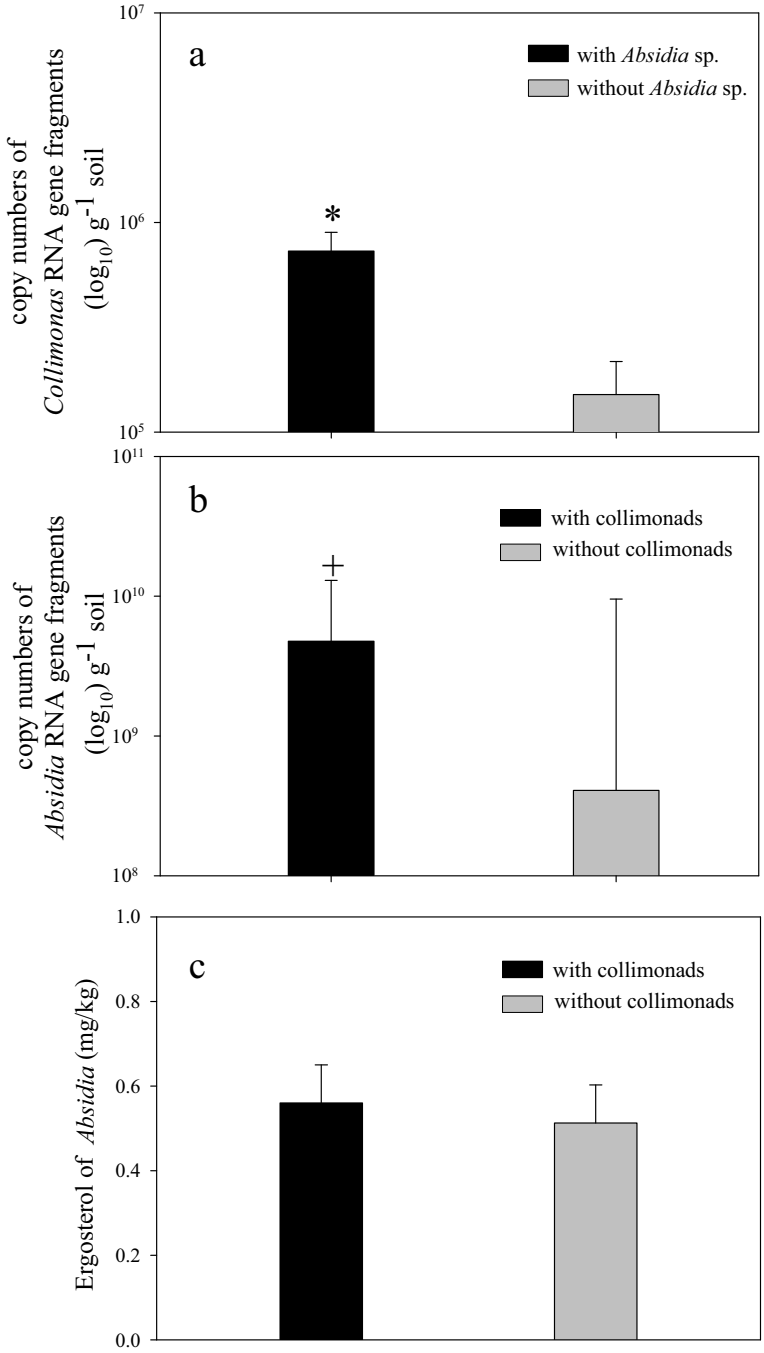

a
with *Absidia* sp.
without *Absidia* sp.
*
copy numbers of *Collimonas* RNA gene fragments (log_{10}) g^{-1} soil
10^7
10^6
10^5
b
with collimonads
without collimonads
+
copy numbers of *Absidia* RNA gene fragments (log_{10}) g^{-1} soil
10^{11}
10^{10}
10^9
10^8
c
with collimonads
without collimonads
Ergosterol of *Absidia* (mg/kg)
1.0
0.8
0.6
0.4
0.2
0.0

FIG 4.2: Growth response of collimonads and the fungus Absidia to each others presence in microcosm containing purified sand. *Collimonads were mixed into the soil and Absidia invaded the soil from a nutrient-rich agar disk. (a) Numbers of 16S rRNA fragments of collimonads, (b) Numbers of 18S rRNA fragments of Absidia, (c) Concentration of the fungal membrane component ergosterol. Data represent the means and standard deviations of six replicates that were harvested 2 and 3 weeks after introduction of the Absidia inoculum. Different symbols within a graph indicate significant differences between single (Absidia or collimonads) and mixed (Absidia and collimonads) treatments. (+ ; $P \leq 0.1$, * ; $P \leq 0.05$, ** ; $P \leq 0.01$) based on Tukey's HSD test.*

Interaction between* Collimonas *and* Trichoderma *mycophagy (experiment 2). The increase of real-time PCR-based numbers of the ITS region of *Trichoderma* rRNA upon introduction of *Absidia* was significantly lower in the presence of collimonads (FIG 4.3). The increase of numbers of collimonads due to invasion of *Absidia* hyphae was not significantly affected by the presence of *T. harzianum*.

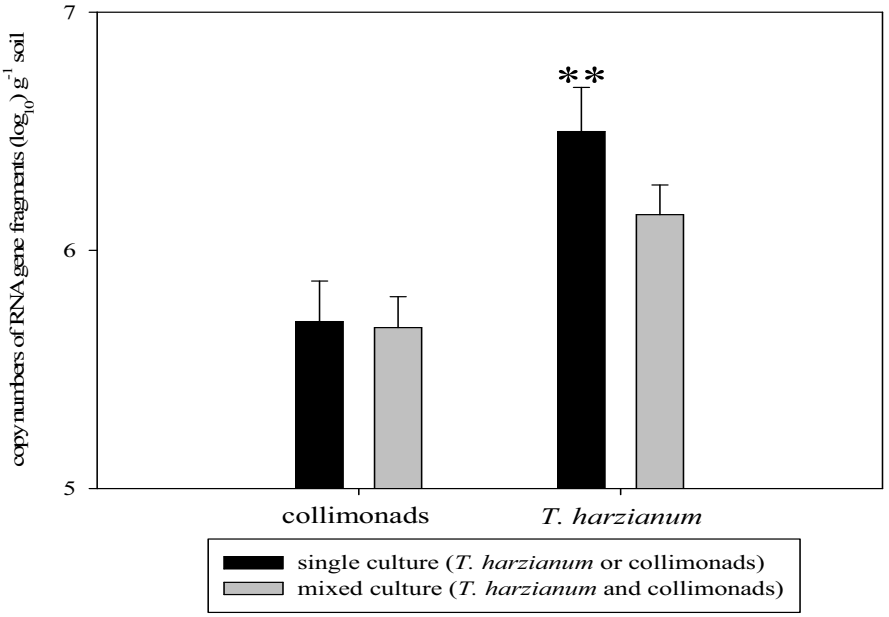


FIG 4.3: Growth response of collimonads and T. harzianum to invading hyphae of the fungus Absidia in microcosms containing purified sand. *Treatments involved the presence of only collimonads, of only T. harzianum or of both collimonads and T. harzianum. Copy numbers of the Collimonas 16S rRNA or Trichoderma ITS region were determined by real-time PCR in the mycelial zone of Absidia 3 weeks after introduction of this fungus. Data represent the means and standard deviations for six replicates that were harvested. Different symbols within a graph indicate significant differences between single (T. harzianum or collimonads) and mixed (T. harzianum and collimonads) treatments. (+ ; $P \leq 0.1$, * ; $P \leq 0.05$, ** ; $P \leq 0.01$) based on Tukey's HSD test.*

DISCUSSION

The increase in numbers of indigenous soil collimonads upon introduction of *Absidia* mycelium indicates that the fungal-induced growth response of collimonads is not restricted to artificial environments (sand microcosms, agar) but can also occur in natural soils. In contrast to collimonads, numbers of bacteria belonging to genera *Pseudomonas* and *Burkholderia* did not increase after the soil had been invaded by *Absidia* hyphae. Several *Pseudomonas* and *Burkholderia* spp. were found to be associated with fungal hyphae, probably growing on fungal exudates [8, 87, 88]. The fact that numbers of bacteria belonging to these genera did not increase upon introduction of *Absidia* hyphae, makes it unlikely that the growth response of collimonads was due to assimilation of exudates. Hence, our investigation points towards an active mycophagous growth by collimonads on *Absidia* hyphae in field soils.

Comparison of bacterial DGGE patterns between control soils and soils subjected to invasion by *Absidia* did not reveal other groups of bacteria that were stimulated by the introduction of the fungus. Hence, it appears that the stimulation of growth of *Collimonas* bacteria by fungal hyphae is not common among other soil bacteria. However, PCR-DGGE analysis does only cover a limited number of dominant bacterial taxa, and the presence of other bacteria with similar growth responses as collimonads can by no means be excluded [89].

Remarkably, the increase of indigenous collimonads upon invasion of *Absidia* hyphae in the field soils was only short-term. Within 3 weeks, numbers had dropped again to the levels of the control for most soils. The same dynamics, i.e. a short-term increase in collimonads numbers, was also found when *Collimonas* strains were inoculated in purified sand and exposed to invading hyphae of different fungal species [7](FIG 4.2a). In the study of De Boer *et al*. (2001) collimonads increased only during the extension of fungal hyphae and shortly thereafter. The subsequent decrease of *Collimonas* CFUs in the study of De Boer *et al* (2001) could have been due to a reduction in cultivability of starving

cells. Such an explanation is unlikely for the current study where the real-time PCR enumeration of collimonads was not dependent on the cultivability of strains. Since the same dynamics of numbers of collimonads, i.e. short-term increase was found for both the indigenous collimonads in field soils and *Collimonas* isolates in pure sand, it is unlikely to be caused by predatory or antagonistic soil microorganisms. The same trend of decrease in real-time PCR based numbers during prolonged incubation was also found for pseudomonads, *Burkholderia* spp. and *Trichoderma* spp. for some of the soils. Increase of inhibition of real-time PCR in the prolonged incubated samples does not offer an explanation as we did not find PCR inhibition in any of our samples (data not shown). Hence, for the time being we cannot explain the apparent decrease of collimonads following the fungal-induced increase.

Whereas there is a significant growth increase of collimonads to invading hyphae in both field soils and pure sand, the absolute amount of bacterial biomass produced at the expense of living fungal hyphae is low : Assuming a cell volume of 0.17 μm^3 and a density of 0.8 g/cm^3 [90], the increase of *Collimonas* biomass in 2 weeks incubation was calculated to be 0.05 µg per g soil. Ergosterol data were used to calculate the fungal biomass. For zygomycetes a conversion factor of 3 mg ergosterol per g biomass can be applied [62]. This implies that the amount of 0.5 µg of ergosterol that was measured per g soil is equivalent to 167 µg *Absidia* biomass. This amount of fungal biomass is 3.3×10^3 times bigger than the 0.05 µg of *Collimonas* biomass produced. So even when we assume a low growth efficiency of the collimonads, the reduction of fungal biomass production by collimonads appears to be negligible. Our observation that biomass production by *Absidia* was not significantly reduced by the presence of collimonads is in line with these calculations. Real-time quantification of 18S rRNA fragments of *Absidia* showed even a trend towards increase in the presence of collimonads, but this trend was not observed with ergosterol measurements. Overall, it can be concluded that the impact of mycophagous collimonads on fungi is small with respect to biomass production but the bacteria may be able to introduce

morphological changes e.g. increased branching. Changes in fungal morphology as a result of the presence of collimonads have been observed by Deveau *et al.*, [91]. They showed that *C. fungivorans* Ter331 decreased the mycelial extension of *L. bicolor* S238N but increased the branching density.

The growth dynamics of the mycophagous *Trichoderma* spp. were examined in a similar way as those of collimonads. Except for site 22, the results of the real-time PCR analyses indicated that copy numbers of *Trichoderma* ITS fragments had increased significantly after 2 weeks of exposure to invasion by *Absidia* hyphae. Thus, the increase of indigenous mycophagous collimonads did not prevent an increased abundance of mycophagous *Trichoderma* spp. and *vice versa*. Yet, the experiments in the gnotobiotic microcosms containing both collimonads and *T. harzianum* indicated that the presence of collimonads can have a negative effect on mycophagous growth of *Trichoderma* spp. (FIG 4.3). This negative effect is most likely caused by antibiosis as resource competition, i.e. competition for *Absidia* hyphae, is not likely given the fact that collimonads do not reduce the fungal biomass of *Absidia* (FIG 4.2). Another explanation might be competition for the preferential zones to attack *Absidia* hyphae. However, whereas the attack by collimonads is probably limited to hyphal tips [7], this is not the case for *Trichoderma* spp. which can penetrate mature hyphae via appressorium formation [92]. There is also the possibility that collimonads grew at the expense of *T. harzianum* hyphae, but tests on water-agar indicated that this is not likely (data not shown). Production of antibiotics by collimonads has been indicated as an important factor in mycophagous behavior but it may also result in inhibition of biomass formation of *Trichoderma* [63].

In conclusion, we showed that indigenous soil collimonads respond to the introduction of *Absidia* hyphae into soil. This supports the importance of mycophagy as a life history characteristic of collimonads. The mycophagous growth of collimonads is of minor importance for fungal biomass dynamics but may affect fungal growth patterns and also community dynamics. In addition, we

showed that collimonads can have a negative effect on the mycophagous growth of *T. harzianum*.

Appendix

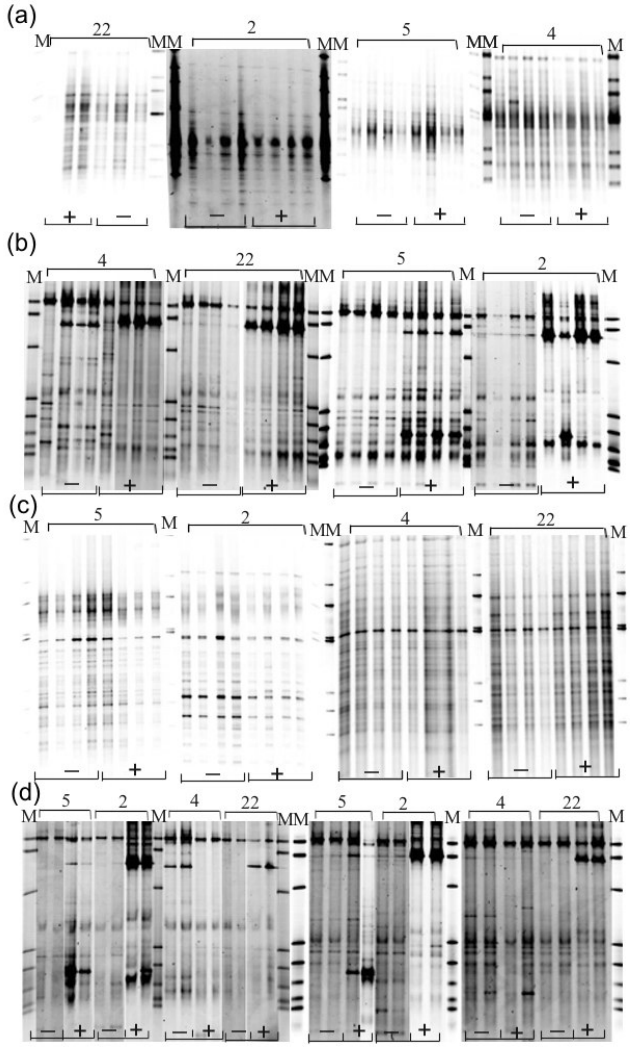


FIG 4.4: *DGGE gels of PCR-amplified fragments of 16S-RNA genes (bacteria) and 18S-RNA genes (fungi) of 4 soils with and without invading hyphae of the fungus Absidia: (a) bacterial PCR-DGGE after 2 weeks (b) fungal PCR-DGGE after 2 weeks (c) bacterial PCR-DGGE after 3 weeks (d) fungal PCR-DGGE after 3 weeks (e) Burkholderia PCR-DGGE after 2 weeks (d) Burkholderia PCR-DGGE after 3 weeks. Sign; + denote samples with invading hyphae of the fungus Absidia. Sign; – denote samples without invading hyphae of the fungus Absidia. Sign; M denote a molecular marker made of an artificial mix of different bacterial/fungal species. Numbers are corresponding to site numbers of 4 soils (site: 2, 4, 5 and 22).*