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ORIGINAL ARTICLE

Mycophagous growth of *Collimonas* bacteria in natural soils, impact on fungal biomass turnover and interactions with mycophagous *Trichoderma* fungi

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Bacteria of the genus *Collimonas* are widely distributed in soils, although at low densities. In the laboratory, they were shown to be mycophagous, that is, 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 the invasion of field soils by fungal hyphae (*Absidia* sp.) resulted in a short-term, significant increase (average fourfold) of indigenous collimonads. No such responses were observed for other soil bacteria studied (*Pseudomonas*, *Burkholderia*, PCR-denaturing gradient gel electrophoresis 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 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 in the *Absidia* biomass. Overall, the study indicates that mycophagous nutrition may be important for collimonads in natural soils, but the impact on fungal biomass turnover is likely to be minor.
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Introduction

Mycophagy, that is, the feeding on living fungi, has been reported for soil bacteria of the genus *Collimonas* (De Boer *et al.*, 2004; Leveau and Preston, 2008). 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 (De Boer *et al.*, 2001). However, whereas the nutrient-poor conditions in these microcosms were realistic for natural soils, other conditions, for example, the absence of other (micro) organisms and plant roots, were different from the natural soil environment.

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In a field inventory, we investigated the distribution of collimonads among different soils (Höppener-Ogawa *et al.*, 2007). 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, that is, they are facultative mycophagous (De Boer *et al.*, 1998).

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 (Lorito *et al.*, 1998; Harman, 2006). Most of these studies deal with *Trichoderma* species (Elad *et al.*, 1983; Donzelli and Harman, 2001). In particular, the application of *Trichoderma* spp. to control plant pathogenic fungi (for example,

Rhizoctonia spp. and *Fusarium* spp.) has received much attention (Hermosa *et al.*, 2000; Whipps, 2001; Harman, 2006). *Trichoderma* spp. are widely distributed in the terrestrial environment (Samuels, 1996). 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 (De Boer *et al.*, 1998). Hence, for both collimonads and *Trichoderma* spp., other sources of energy, for example, 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.

Materials and methods

Soils

In January 2007, soil samples (upper 10 cm of mineral layer) were collected from sites where collimonads had been detected earlier (Höppener-Ogawa *et al.*, 2007). The soils were a forest soil (site 2), two grassland soils (sites 4 and 5) and a soil from an abandoned arable site (site 22), respectively. Characteristics of these soils (sites 2, 4, 5 and 22) were described earlier 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. (Höppener-Ogawa *et al.*, 2007).

Bacterial strains

The strains used in this study were *Collimonas fungivorans* Ter 331 (AJ310395) and Ter 6^T (LMG 21973), *Collimonas arenae* Ter 10^T (LMG 23964) and *Collimonas pratensis* Ter 91^T (LMG 23965) (De Boer *et al.*, 2004; Höppener-Ogawa *et al.*, 2008). These 4 strains have been isolated from the same soil (De Boer *et al.*, 2004). Hence, their co-occurrence is natural. Based on the experimental conditions and feeding-preferences, the mixture of the four 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 earlier (De Boer *et al.*, 2007). *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

On the basis of earlier study, it was evident that the growth of collimonads can be especially stimulated by zygomycetal fungi (De Boer *et al.*, 2001). In the current study, we used a zygomycetal fungus, isolated from a grassland soil, that was identified as *Absidia* sp. on the 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 (Gardes and Bruns, 1993). *Absidia* spp. are common saprotrophic soil fungi (Hoffmann *et al.*, 2007). *Trichoderma harzianum* CECT 2413 (Rubio *et al.*, 2005) 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 (40 g) 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 performed in sixfold.

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-denaturing gradient gel electrophoresis (PCR-DGGE) analysis of the bacterial community structure were performed to evaluate the specificity of the growth increase in 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, USA) 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 the 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*. (2001). *Absidia* sp. was grown on PDA at 20 °C for 4 days.

The four different *Collimonas* strains were mixed by adding equal number 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 per gram sand (based on microscopic counts), which is a common density of collimonads in field soils (Höppener-Ogawa *et al*., 2007). 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 microorganisms 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 the measurement of fungal biomass (ergosterol) and the abundance of collimonads (real-time PCR).

The same experimental setup was used to the study interactions of collimonads with the mycophagous fungus *T. harzianum* during growth on *Absidia* hyphae.

Sterile sand with or without collimonads (see above) was inoculated with a spore suspension (10^4 spores per gram 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 r.p.m. for 1 min. 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 per gram sand (based on microscopic counts). All treatments were performed in sixfold. Using the prepared microcosms in the same experimental setup 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 the measurement of the 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, NSW, Australia). All mixes were made using a CAS-1200 pipetting robot (Corbett Research) to reduce variation caused by pipetting errors. Quantification of collimonads in soil DNA samples was performed as described by Höppener-Ogawa *et al*. (2007).

For quantification of pseudomonads, *Burkholderia* spp. and *Trichoderma* spp., the Absolute qPCR SYBRGreen mixture (ABgene, Epsom, UK) 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 *P. fluorescens* strain AD21 and *Burkholderia* JS (see strain description). The *Pseudomonas*-specific primer sets used were PSf (5′-GGTCTGAGAGGATGATCAGT-3′) and PSr (5′-TTAGCTCCACCTCGCGGC-3′) (Widmer *et al*., 1998). The *Burkholderia*-specific primer sets used were Burk3 (5′-CTGCGAAAGCCGGAT-3′) and BurkR (5′-TGCCATACTCTAGCYYGC-3′) (Salles *et al*., 2002).

For quantification of *Trichoderma* spp., we used a modified protocol of Hagn *et al*. (2007). The *Trichoderma* standard curve was made from the DNA extracted from a pure culture of *T. harzianum* CECT 2413. PCR amplification was performed using the fungal universal primer sets NS1 (5′-GTAGTCAT ATGCTTGTCTC-3′) (White, 1990) and TW13 (5′-GG TCCGTGTTTCAAGACG-3′) (Taylor and Bruns, 1999).

For quantification of *Absidia* sp., real-time PCR quantification was performed using universal fungal-specific primers as described elsewhere (Vainio and Hantula, 2000). In addition, measurement of the fungal cell membrane component ergosterol by an alkaline extraction protocol was performed to quantify the biomass of *Absidia* sp. (De Ridder-Duine *et al*., 2006).

PCR-DGGE analysis

All PCR reactions and DGGE were carried out as described elsewhere (Salles *et al*., 2002; Yergeau *et al*., 2007). The primers 968f-GC and 1378r (Heuer *et al*., 1997), and FR1-GC and FF390r (Vainio and Hantula, 2000) were used to analyze bacterial and fungal communities, respectively. The nested PCR reactions were performed for *Burkholderia* spp. with primers Burk3 and R1378 (Salles *et al*., 2002). The products from the first PCR were diluted at 1:1000 and used as the template in the second PCR with primers Burk3-GC and BurkR (Salles *et al*., 2002).

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 (Legendre and Anderson, 1999). Jaccard's coefficient of similarity was calculated and the resulting similarity matrix was exported to Canoco 4.5 as species data for redundancy analysis (Ter Braak and Šmilauer, 2002). 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, whereas both individual factors were included (without interaction) as co-variables. The significances of such models were tested with 999 permutations.

All analyses of variance were performed using Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA). For analysis of variance, normal distribution of data was tested with the 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 the introduction of *Absidia* mycelium on the number of collimonads is shown in Figures 1a and b. The real-time PCR-based numbers of indigenous collimonads in the soils were 5.0×10^4 per gram soil for sites 2, 4 and 5, and 7.5×10^5 per gram soil for the site 22. These numbers did not increase during 2 weeks of incubation without the introduction of fungal hyphae. The numbers of collimonads had increased (4.1-fold on average) in all four soils after 2 weeks of invasion by *Absidia* hyphae. However, with the exception of soil for the site 4, the stimulating effect of invasion by *Absidia* hyphae was no longer apparent when the incubation period was 1 week longer (3 weeks in total).

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

Real-time PCR-based numbers of indigenous fungi belonging to the genus *Trichoderma* were significantly increased in two soil sites (2 and 5) after 2 weeks of the introduction of *Absidia* (Figures 1g and h). In the soil of site 4, the increase was significant at the level of $P<0.1$. The average increase in real-time PCR-based numbers in these three soil sites was 26.8-fold. After 3 weeks of incubation, a significant stimulation of the abundance of *Trichoderma* spp. was only found for the soil site 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 distance-based RDA (Table 1, Supplementary Table S1). The bacterial community structure, including *Burkholderia* community structure, appeared to be unchanged, whereas the fungal community structure was significantly influenced by the introduction of *Absidia* in all the soils that we tested. For all soils, the band corresponding to *Absidia* was clearly visible in the gel.

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 in the real-time PCR-based numbers of collimonads in the mycelial zone (Figure 2a). The dynamics of the abundance of *Absidia* was assessed by quantification of 18S rRNA gene copies and ergosterol (Figures 2b and c, 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 microcosms 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.

Interaction between Collimonas *and* Trichoderma *mycophagy (experiment 2)*

The increase in the real-time PCR-based numbers of the ITS region of *Trichoderma* rRNA upon introduction of *Absidia* was significantly lower in the presence of collimonads (Figure 3). The increase in the numbers of collimonads because of the invasion of *Absidia* hyphae was not significantly affected by the presence of *T. harzianum*.

Discussion

The increase in the 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, the numbers of bacteria belonging to genera *Pseudomonas* and *Burkholderia* did not increase after the soil

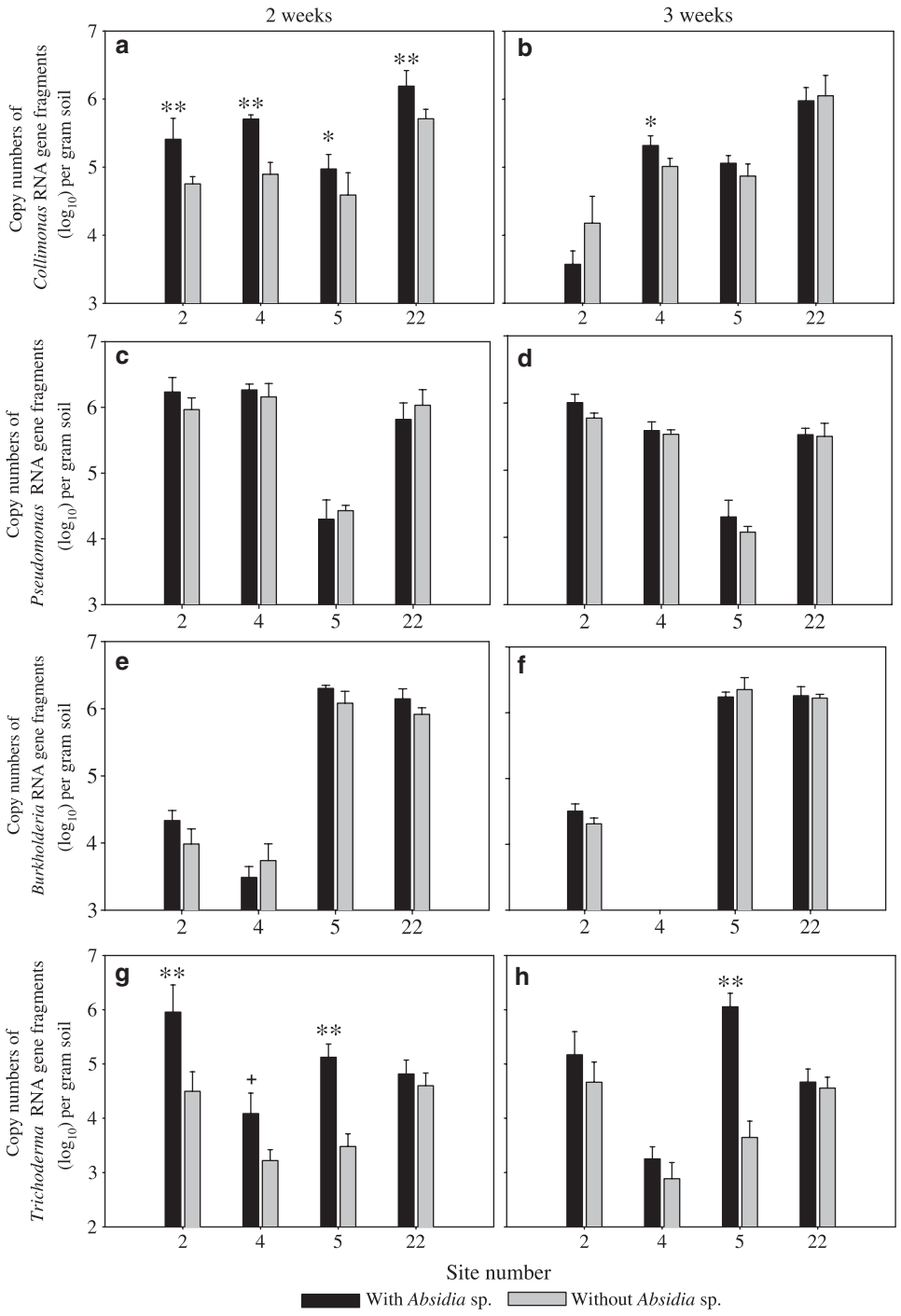

Figure 1 Growth response of indigenous soil bacteria belonging to the genus *Collimonas* (**a** and **b**), *Pseudomonas* (**c** and **d**), *Burkholderia* (**e** and **f**) and of indigenous soil fungi belonging to the genus *Trichoderma* (**g** and **h**) upon extension of mycelium of the fungus *Absidia* in four field soil microcosms (sites 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 the introduction of *Absidia* (gray bar). Real-time PCR-based quantifications were performed 2 and 3 weeks after the introduction of fungal inoculum. Data represent the means and s.d. 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 the introduction of *Absidia* ($^{+}P \leqslant 0.1$, $^{*}P \leqslant 0.05$ and $^{**}P \leqslant 0.01$) based on Tukey's HSD test.

had been invaded by *Absidia* hyphae. Several *Pseudomonas* and *Burkholderia* spp. were found to be associated with fungal hyphae, probably growing on fungal exudates (Singh and Arora, 2001; De Boer *et al.*, 2005; Izumi *et al.*, 2008). The fact that the 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 the assimilation of exudates. Hence, our investigation points toward

Table 1 Distance-based redundancy analyses 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 four different field soils

Incubation time	*Factors*	*Bacteria*		*Fungi*	
		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 origin	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 origin	0.071	1.000	2.728	0.001

Abbreviation: PCR-DGGE, PCR-denaturing gradient gel electrophoresis.

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 *Collimonas* bacteria growth by fungal hyphae is not common among other soil bacteria. However, the 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 (Kowalchuk *et al*., 2004).

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, that is 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 (De Boer *et al*., 2001; Figure 2a). In the study by De Boer *et al*. (2001), collimonads increased only during the extension of fungal hyphae and shortly thereafter. The subsequent decrease in *Collimonas* colony forming units (CFU) in the study by 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. As the same dynamics of the numbers of collimonads, that is 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 in the 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. The increase in the 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 in collimonads after the fungal-induced increase.

Although there is a significant growth increase in 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 $g\,cm^{-3}$ (Van Veen and Paul, 1979), the increase in *Collimonas* biomass in 2 weeks of incubation was calculated to be 0.05 μg per gram soil. Ergosterol data were used to calculate the fungal biomass. For zygomycetes, a conversion factor of 3 mg ergosterol per gram biomass can be applied (Olsson *et al*., 2003). This implies that the amount of 0.5 μg of ergosterol that was measured per gram 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 toward an 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, for example increased branching. Changes in fungal morphology as a result of the presence of collimonads have been observed by Deveau *et al*. (2007). They showed that *C. fungivorans* Ter 331 decreased the mycelial extension of *L. bicolor* S238N but increased the branching density.

The growth dynamics of the mycophagous *Trichoderma* spp. was examined in a similar way as that of collimonads. Except for the 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

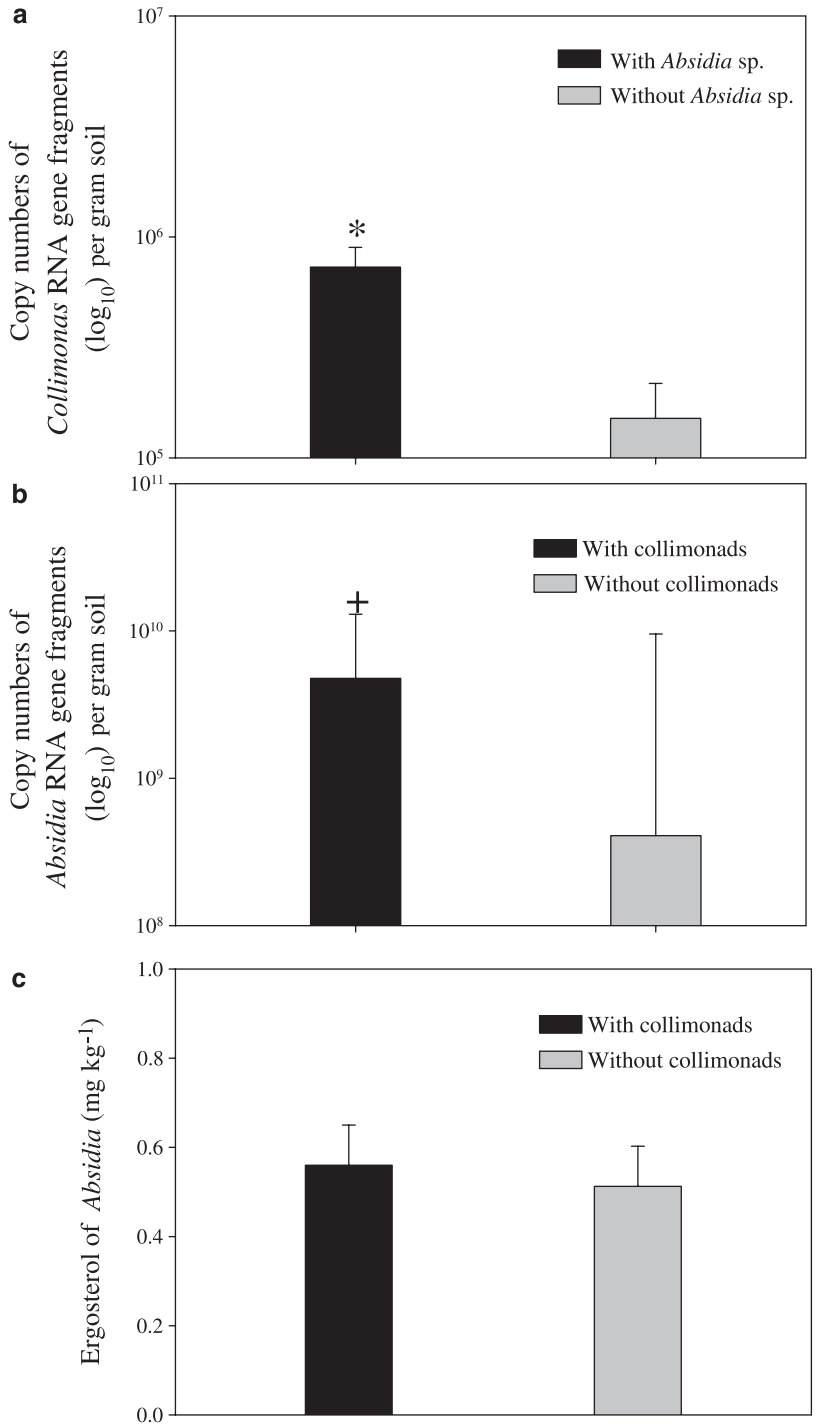

Figure 2 Growth response of collimonads and the fungus *Absidia* to each other's presence in microcosms containing purified sand. Collimonads were mixed into the soil, and *Absidia* invaded the soil from a nutrient-rich agar disk. (**a**) The numbers of 16S rRNA fragments of collimonads, (**b**) the numbers of 18S rRNA fragments of *Absidia* and (**c**) concentration of the fungal membrane component ergosterol. Data represent the means and s.d. of six replicates that were harvested 2 and 3 weeks after the introduction of the *Absidia* inoculum. Different symbols within a graph indicate significant differences between single (*Absidia* or collimonads) and mixed (*Absidia* and collimonads) treatments ($^{+}P \leqslant 0.1$ and $^{*}P \leqslant 0.05$) based on Tukey's HSD test.

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.

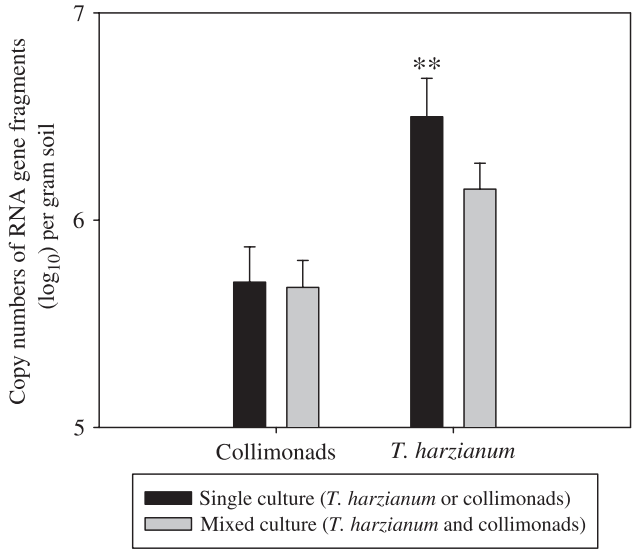

Figure 3 Growth response of collimonads and *Trichoderma harzianum* to invading hyphae of the fungus *Absidia* in microcosms containing purified sand. Treatments involved the presence of only collimonads, of only *T. harzianum* or of both collimonads and *T. harzianum*. Copy numbers of the *Collimonas* 16S rRNA or *Trichoderma* internal transcribed spacer (ITS) region were determined by real-time PCR in the mycelial zone of *Absidia* 3 weeks after the introduction of this fungus. Data represent the means and s.d. 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 ($^{**}P \leqslant 0.01$) based on Tukey's HSD test.

(Figure 3). This negative effect is most likely caused by antibiosis, as resource competition, that is competition for *Absidia* hyphae, is not likely given the fact that collimonads do not reduce the fungal biomass of *Absidia* (Figure 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 (De Boer *et al.*, 2001), this is not the case for *Trichoderma* spp., which can penetrate mature hyphae by the appressorium formation (Inbar and Chet, 1994). 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 the inhibition of the biomass formation of *Trichoderma* (Höppener-Ogawa *et al.*, 2007).

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*.

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