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ORIGINAL PAPER

Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization

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Abstract Soil organic matter is known to influence arbuscular mycorrhizal (AM) fungi, but limited information is available on the chemical components in the organic matter causing these effects. We studied the influence of decomposing organic matter (pure cellulose and alfalfa shoot and root material) on AM fungi after 30, 100, and 300 days of decomposition in nonsterile soil with and without addition of mineral N and P. Decomposing organic matter affected maize root length colonized by the AM fungus *Glomus claroideum* in a similar manner as other plant growth parameters. Colonized root length was slightly increased by both nitrogen and phosphorus application and plant materials, but not by application of cellulose. In vitro hyphal growth of *Glomus intraradices* was increased by soil extracts from the treatments with all types of organic materials independently of mineral N and P application. Pyrolysis of soil samples from the different decomposition treatments revealed in total 266 recognizable organic compounds and in vitro hyphal growth of *G. intraradices* in soil extract positively correlated with 33 of these compounds. The strongest correlation was found with 3,4,5-trimethoxybenzoic acid methyl ester. This compound is a typical product of pyrolysis of phenolic compounds produced by angiosperm woody plants, but in our experiment, it was produced mainly from cellulose by some components of the soil microflora. In conclusion, our results indicate that mycelia of AM fungi are influenced by organic matter decomposition both via compounds released during the decomposition process and also by secondary metabolites produced by microorganisms involved in organic matter decomposition.

Keywords Pyrolysis · Biomarker fatty acid · 3,4,5-Substituted benzyl structures · Humus

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Introduction

Arbuscular mycorrhizal (AM) fungi represent an important component of the soil microbial community that can significantly affect plant growth and soil stability. Since soil is supplied with decomposing organic materials of various origin, mycelium of soil inhabiting AM fungi is in permanent contact with complex products of decomposition possessing considerable biological activity.

Despite the key role of organic matter in soil ecosystems (Lejon et al. 2007), limited information on the effect of soil organic matter on AM fungi is available. However, it is well known that growth of AM fungi can be both increased (Hepper and Warner 1983; StJohn et al. 1983; Joner and Jakobsen 1990; Rydlová and Vosátka 2000; Gryndler et al.

2002; Gryndler et al. 2006; Albertsen et al. 2006) and decreased (Avio and Giovannetti 1988; Calvet et al. 1992; Ravnskov et al. 1999; Ravnskov et al. 2006) by soil organic amendments.

Cellulose is the most abundant component of organic matter entering soil from decaying plant materials. Pure cellulose can increase root colonization and external mycelial growth of AM fungi but only after it has been sufficiently decomposed. (Gryndler et al. 2002). However, if cellulose is used fresh or composted for shorter periods, it can inhibit mycorrhizal symbiosis (Avio and Giovannetti 1988; Ravnskov et al. 1999; Gryndler et al. 2002).

In general, studies on the influence of organic matter on AM fungi have provided inconsistent results indicating variable effects of different organic substrates on mycorrhizal symbiosis. All the aforementioned studies were treating the decomposition of the organic amendments as a black box, failing to describe the link between the growth of AM fungi and a cascade of organic compounds released during the decomposition process.

The objective of the present work was to examine the influence of organic matter decomposition on AM fungi, linking soil organic compounds generated by the decomposition process, as examined using soil pyrolysis, with the growth of hyphae of AM fungi and colonization of a host plant. Also, microbial biomarker fatty acids were employed to link different groups of microorganisms with soil organic compounds. The main hypothesis was that organic matter increases the asymbiotic hyphal growth of the AM fungus due to specific compounds produced during organic matter decomposition.

Materials and methods

Experimental design

The experiment had a randomized design, and the treatments were combinations of three factors, including four levels of organic material [unamended control (1), amended with microcrystalline cellulose Sigmacell 20-Sigma S3504 (2), amended with field-collected dry alfalfa roots (3), or amended with dry alfalfa shoots (4)], two levels of mineral nutrition [control with no minerals applied (1) and soil supplied with 139 mg N, 103 mg P, and 516 mg k kg^{-1} (2)], and three levels of length of decomposition period [30 days (1), 100 days (2), and 300 days (3)]. Each treatment had four replicates giving a total of 96 experimental units.

Setup of decomposition experiment

Nonsterile soil (tilled layer of Orthic Luvisol, clay–loam, developed on dilluvial sediments mixed with loess, pH 6.5) was collected at the experimental field of the Institute of Crop Production, Prague, Czech Republic. The soil was sieved through 5 mm, and an aliquot was taken to measure the moisture content. Minerals were added as KNO_3 and KH_2PO_4 dissolved in water to half of the experimental units. Organic additives were first pulverized in a blender and added at a rate of 1% (*w*/*w*) into the air-dried soil. Field-collected alfalfa roots and shoots were dried for 10 h at 75°C before being pulverized. This treatment eliminated living propagules of AM fungi present in the roots. Alfalfa shoots contained 3.2 g P and 28 g N per kg, alfalfa roots contained 3.5 g P and 19 g N per kg. The treated soil samples were then distributed to 500 ml vented plastic vessels so that each vessel finally contained 200 g soil with 25% moisture. The vessels were incubated in the dark at 20°C, and soil moisture content (25%) was controlled gravimetrically. Vessels with soil were harvested after 30, 100, and 300 days of incubation. The soil in each vessel was homogenized and stored at −20°C in sealed plastic sacs until further use.

Effect of water soluble soil extracts on in vitro AM hyphal growth

Sample aliquots prepared from the soil harvested from each of the 96 experimental units of the above-described decomposition experiment were used to prepare water extracts of soil. The extracts were prepared from 50 g soil added to 50 ml deionized water and shaken (vertical shaker, frequency 5 Hz, amplitude 3 cm) for 30 min in a 250-ml screw-capped bottle. The resulting suspension was centrifuged for 10 min at 15,000×*g* to remove soil particles. Absorbance at 280 nm and pH of the supernatant (soil extract) were then measured.

Effect of soluble products of composted material on the growth of hyphae in vitro was assayed in an experimental system previously developed for *Glomus intraradices* as a test organism (Malcová et al., 2002). Roots colonized by *G. intraradices* (isolate PH5, Institute of Botany, CAS, Průhonice, Czech Republic) were obtained from a hydroponic system with maize as a test plant, cultivated in plastic tubes (18 cm in height and 5 cm in diameter) filled with perlite as a carrier material and covered with canvas at the bottom. Each tube was located in a black plastic vessel and supplied once a week by 300 ml nutrient solution P2N3 (Gryndler et al. 1992) containing 0.1 mM phosphate. Two pregerminated maize seeds were inoculated in each tube with approximately 20 g inoculum of the AM fungus and cultivated for 6 weeks under constant environmental conditions (16 h photoperiod, 450 μmol photons m^{-2} s^{-1}, 23°C/70% r. h. day, 18°C/75% r. h. night). Sixteen tubes (32 plants) were harvested. Root systems were washed, and bright yellow mycorrhizae were collected and superfi-

cially decontaminated for 4 h in a solution of antibiotics (500 mg/l penicillin G, 500 mg/l streptomycin, 500 mg/l neomycin, 500 mg/l polymyxin B and 250 mg/l rolitetracycline). The decontamination procedure was completed by immersion of roots in a 6% solution of sodium hypochlorite diluted 1:50 for 3 min and washing with 1 l sterile deionized water. The roots were then cut into approximately 2-mm long segments with surgical scissors. The segments were transferred to 30 µl drops of filter-sterilized incubation medium placed inside inverted plastic Petri dish lids, covered with matching dishes and re-inverted again. Each Petri dish (representing one out of the three dishes established per replicate) contained 16 drops of the filter-sterilized undiluted extract of soil, each drop containing 1 mycorrhizal root segment. Before filter-sterilization, pH values of extracts were always adjusted to 6.3 using 0.1 M KOH/HCl.

Petri dishes were incubated in a humid chamber at 25°C for 5 days. After the incubation, the root segments placed in the drops of the incubation medium were observed (through the lid of the dish) under a microscope (magnification ×63) with an eyepiece equipped with a grid net focal plate. The intersections of grid net lines with the mycorrhizal hyphae were counted as a measure of hyphal growth (Newman 1966). The data were expressed as a mean of total length of hyphae per root segment over three Petri dishes (48 root segments in total) per replicate. Only root segments free of contamination with saprotrophic microorganisms were used in the analyses.

Effect of decomposition on AM fungal colonization of maize roots

A plant assay based on the same hydroponic cultivation system as described above was used to examine the influence of the decomposition process on AM fungal root colonization. Twenty-gram sample aliquots of the soil (25% moisture content) harvested from each of the 96 experimental units of the decomposition experiment were steamed in sealed plastic sacs at 67°C for 6 h to inactivate indigenous AM fungi and left to vent in open air at 3°C for 3 days. Three maize plants (cv. "Arobase") were planted per tube, and 20 g tested soil sample inoculated with 300 washed spores of *Glomus claroideum* BEG23 was placed 3 cm under the level of seeds (approximately 5 cm above the maximum level of the nutrient solution). Tubes were then put into plastic tubs and supplied with a mineral nutrient solution P2N3. Each tub contained 14-planted tubes and a volume of 2 l of the nutrient solution. Soil from each replicate was incubated in one separate tube. In total, seven tubs containing 96 soil samples in plastic tubes were established. Nutrient solution was renewed once a week. The described hydroponic cultures were kept for 6 weeks under the same environmental conditions as described in a previous section. Roots were then washed to remove perlite and soil, and aliquots were digested for 30 min in 10% KOH at 90°C, washed again, acidified in 4% lactic acid, and stained in 0.1% Trypan blue in lactoglycerol. Total root length and percentage of root length colonized with AM fungi was then measured using the grid-line intersect method (Giovannetti and Mosse 1980, Newman 1966). In addition, shoot dry weight was recorded.

Whole cell fatty acids analysis of soil

Concentrations of signature whole-cell fatty acids (WCFA) were measured as estimates of the abundance of main groups of soil microorganisms in the sample aliquots prepared from soil harvested from the decomposition experiment. Immediately after the incubation, 25 g freeze–dried sample aliquots from each of the 96 experimental units were pulverized in a steel mill, and 1 g of the resulting powder was subjected to lipid extraction and further analyzed for the content of fatty acids as described by Thygesen et al. (2004). Analyses of fatty acid methyl esters were performed using the software package Sherlock 3.1 (MIDI, Newark, DE, USA) with a HP Chemstation (Hewlett Packard, CA, USA) and a HP5890 GC fitted with a 25-m fused silica capillary column (HP part no. 19091B-102) and hydrogen as carrier gas. The injector and detector temperatures were set to 250°C and 300°C, respectively. The column temperature was programmed so that it increased from an initial 170°C to 270°C at a rate of 5°C/min. One microliter of sample preparation was injected. Calibration standards contained a mixture of straight chain saturated and hydroxylated fatty acid methyl esters with a length of ten to 20 carbon atoms (MIDI, Part No. 1200A).

Pyrolysis analysis of soil

Two pulverized soil sample aliquots per treatment of the decomposition experiment (48 samples in total) were methylated in situ with tetramethyl ammonium hydroxide (TMAH, Sigma) based on the method of Hatcher and Clifford (1994). The samples (1 mg each) were dispersed in 25% solution of TMAH in ratio 1:1 (*w*:*w*), placed on tungsten wire spirals, dried in a desiccator overnight, and inserted into a coil of platinum filament of the PYR-01 pyrolyzer (Labio, Czech Republic). Pyrolysis was performed at 550°C for 10 s in the injector port (240°C) of a gas chromatograph (Varian 3400). The GC instrument was equipped with split injector, and DB-5MS column was used for separation (30 m, 0.25 mm internal diameter, 0.25 µm film thickness, helium as a carrier gas). The temperature program started at 45°C, and the oven was heated to 260°C at a rate of 5°C/min. The final temperature was held for an additional 10 min, and solvent delay time was set to 2 min.

The temperature of transfer line to mass spectrometer (ITS 40, Finnigan) was set to 280°C. Mass spectra were recorded at 1 scan s^{-1} under electron impact at 70 eV and mass range 50–450. Identification of pyrolysis products was performed by comparing the mass spectra with the data in the NIST 02 library, comparison with literature data, and independently by interpreting the fragmentation pattern. Probable origin of the pyrolysis products was assessed from their chemical nature (Steffen et al. 2007).

Data analysis

The data from hyphal growth experiments were subjected to lognormal transformation and analyzed by three-way ANOVA and Duncan's multiple range test, $P \leq 0.05$. The data on root length, root length colonized by the mycorrhizal fungus, and shoot dry weight in maize hydroponic experiment were analyzed by two- and three-way ANOVA and Duncan's multiple range test, $P \leq 0.05$. Besides ANOVA, the correlation coefficients were calculated to evaluate the possible association of growth of hyphae of *G. intraradices* with the strength of signals of soil pyrolysis products in mass detector and to detect association between signal strengths of pyrolysis products and abundance of signature WCFA in soil samples.

Relationship between abundance of pyrolysis products (explaining variables) and parameters describing the absorbance and pH of soil extracts, AM fungal growth, plant mycorrhizal colonization, and plant growth (explained variables) were tested using the redundancy analysis (RDA), using Canoco 4.5 software (Biometris, Wageningen, The Netherlands).

The explained variables (called "species" in Canoco terminology) were square-root transformed, centered, and standardized. Experimental factors (organic amendment, mineral amendment, and sampling time) were exploited as a source of additional variability in abundance of soil organic matter components. Significance of the relationship between explaining and explained variables was assessed using Monte Carlo permutation test (reduced model, 499 permutations).

Results

Effects of decomposition products from alfalfa roots and shoots or cellulose on AM fungi

Extracts of soil samples amended with organic matter significantly affected the growth of *G. intraradices* in vitro, and the effects observed differed according to the length of the decomposition period as revealed by three-way ANOVA (Table 1). Extracts obtained from soil samples after 300 days of incubation tended to decrease hyphal growth of *G. intraradices* in vitro (Fig. 1). No perceptible effect of mineral N and P supply on this parameter was noted. All types of decomposing organic matter increased hyphal growth similarly.

Three-way analysis of variance revealed that organic amendment affected colonized root length (Table 1), and Duncan's multiple range test showed that this parameter was increased if the soil was amended with dead alfalfa tissues. Pure cellulose had negative effect on root colonization (Fig. 2). Testing the ability of *G. claroideum* to colonize roots in hydroponic culture in the presence of the studied soil samples revealed that the incubation of organic materials for 100 days produced the highest value of the root length colonized, whereas the value obtained with soil samples tested after 300 days of incubation was significantly lower than that obtained with soil incubated for 30 days (Fig. 2). Addition of mineral N and P increased root length colonized by the mycorrhizal fungus (Fig. 2).

An interaction between the effects of sampling time and organic amendments on maize root length colonized was observed (Table 1). However, interactions between these factors were not observed if data for unamended soil were excluded from the analysis and analyzed separately. The

Table 1 Results of 3-way variance analysis of effects of the main experimental factors on AM fungi and maize plant growth in hydroponic culture

Parameters	Sources of variability						
	Sampling (A)	Minerals (B)	Organics (C)	Interactions			
				A×B	A×C	B×C	A×B×C
Log HG	6.491 (0.0026)	0.002 (0.9679)	9.520 (<0.0001)	0.178 (0.8370)	2.178 (0.0603)	1.118 (0.3476)	0.612 (0.7197)
Shoots	0.735 (0.4832)	22.578 (<0.0001)	4.935 (0.0036)	0.135 (0.8740)	2.716 (0.0196)	1.750 (0.1644)	0.967 (0.4536)
RL	6.550 (0.0024)	11.802 (0.0010)	15.839 (<0.0001)	0.733 (0.4840)	2.373 (0.0378)	1.218 (0.3094)	1.012 (0.4245)
RLI	32.460 (<0.0001)	12.341 (0.0008)	58.678 (<0.0001)	0.832 (0.4395)	16.083 (<0.0001)	1.160 (0.3309)	1.071 (0.3882)

The main factors are: sampling time (Sampling), mineral nutrients supply (Minerals) and organic amendment (Organics). Effects of these factors on the growth of hyphae (LogHG) of *G. intraradices* in vitro, maize shoot dry weight (Shoots), root length (RL), and root length colonized by *G. claroideum* (RLI) are evaluated. Corresponding *F* values are accompanied by *P* values in parentheses.

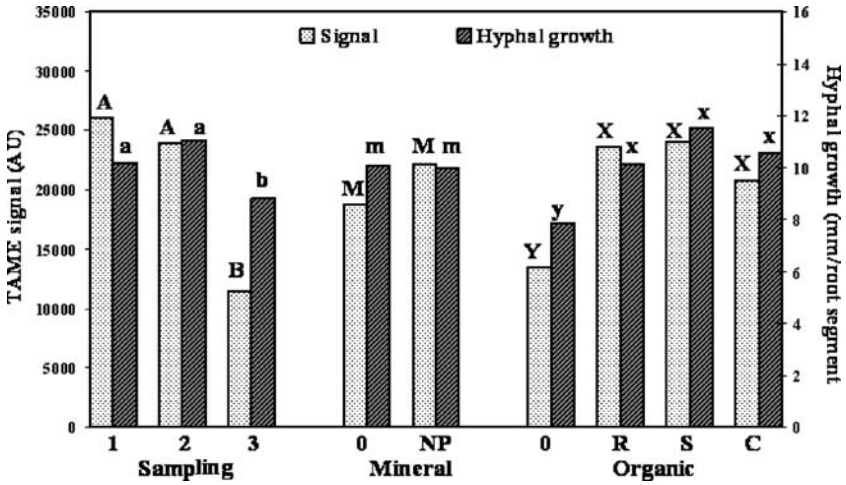

Fig. 1 Effect of time of soil incubation (samplings: *1* 30 days, *2* 100 days, and *3* 300 days), mineral nutrients added to soil samples (*0* none, *NP* nitrate and phosphate applied), and organic additives incubated in the soil samples (*0* none, *R* root tissues, *S* shoot tissues, *C* purified cellulose) on growth of hyphae of *G. intraradices* in vitro and 3,4,5 trimethoxybenzoic acid methyl ester (TAME) abundance in soil pyrolysis products. TAME signal is expressed in arbitrary units of mass detector response (AU). The *same letters associated with columns* within a main effect set indicate that the mean values presented by the columns do not differ significantly at $P \leq 0.05$. *Uppercase and lowercase letters* are valid for signal and hyphal growth data, respectively. Data were evaluated by three-way ANOVA and Duncan's multiple range test and sorted according to the factors under study

results indicate that prolonged incubation of unamended soil samples had no effect on mycorrhizal root colonization, unlike the 100-day incubation of amended samples, which resulted in increased root colonization compared to samples incubated for 30 days only (Fig. 3).

Effects of decomposing alfalfa tissues or cellulose on maize growth in hydroponic culture

Organic amendment affected total root length (Table 1) and Duncan's multiple range test showed that this parameter was increased if the soil was amended with decomposing alfalfa roots and shoots (Fig. 2). Maize shoot dry weight was positively affected only in the treatment amended with shoot tissues. Interactions between sampling time and organic amendments were found regarding their effect on the parameters' root length and shoot weight (Table 1). The data from unamended soil were thus excluded from the further analysis and analyzed separately from amended samples (Fig. 3). When compared with soil samples incubated for 30 days only, the unamended soil samples incubated for 100 days significantly decreased shoot dry weight of maize, whereas the amended samples behaved oppositely. Further, amended soil samples incubated for 100 days increased the total root length when compared to the amended samples incubated for 30 days only.

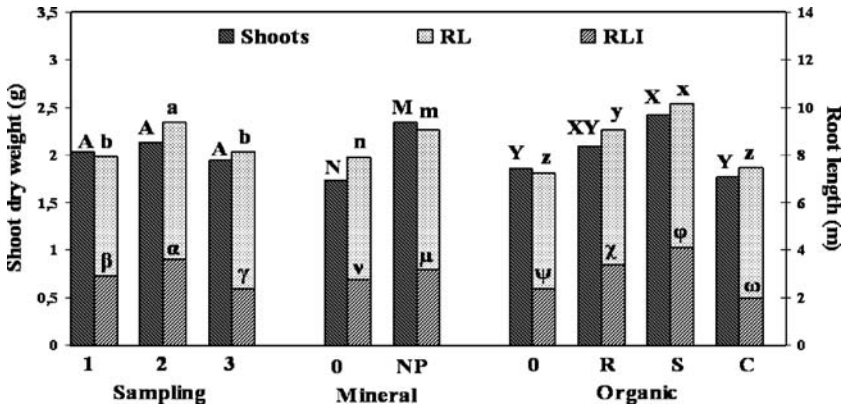

Fig. 2 Effect of time of incubation, mineral nutrients, and organic additives on growth of maize in hydroponic culture and on mycorrhizal root colonization. For explanation of symbols see Fig. 1. Data on shoot dry weight (shoots), root length (RL), and portion of roots colonized by *G. claroideum* BEG23 (RLI) are presented. Data were evaluated by three-way ANOVA and Duncan's multiple range test and sorted according to the factors under study. The same letters associated with columns within a main effect set indicate that the mean values presented by the columns do not differ significantly at $P \leq 0.05$. *Uppercase*, *lowercase*, and *Greek letters* are valid for shoot dry weight, root length, and colonized root length, respectively

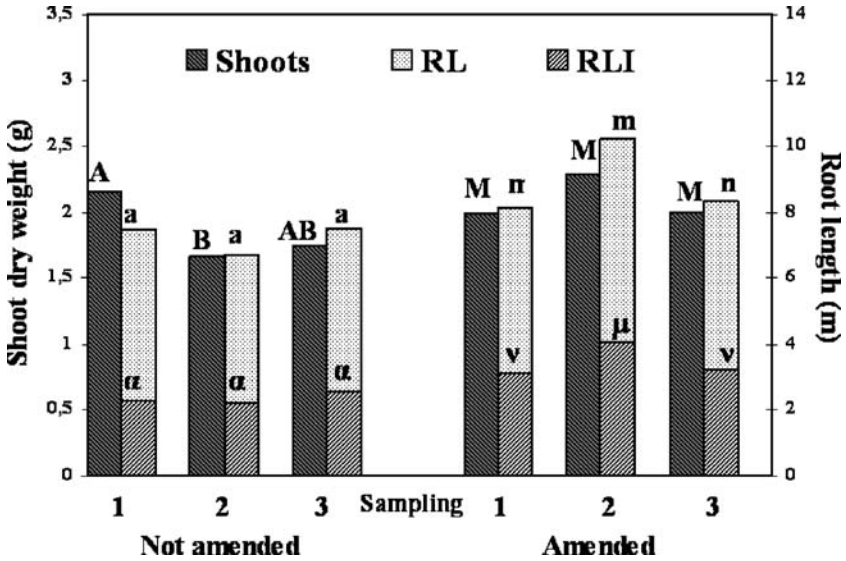

Fig. 3 Effect of sampling time on maize shoot dry weight, root length, and portion of roots colonized by *G. claroideum* BEG23. For explanation of symbols see Figs. 1 and 2. Data were evaluated by two-way ANOVA (unamended samples) or three-way ANOVA (amended samples) and Duncan's multiple range test and sorted according to the sampling time. Other explanations as for Fig. 2

Correlation between plant and AM fungal growth and pyrolysis products

G. intraradices hyphal growth in water extracts from soil samples from the different treatments correlated with pyrolysis products from the corresponding soil samples from which the extracts were obtained. Of the 266 recognizable pyrolysis products found, 33 compounds correlated positively, and one compound correlated negatively with hyphal growth (Table 2). The strongest correlation was found in the case of 3,4,5-trimethoxybenzoic acid methyl ester (TAME, R=0.5431, code 25 in Fig. 5). This correlation was stronger than the correlation of hyphal growth with absorbance of soil extract at 280 nm (R=0.3046).

Since TAME was more strongly correlated with hyphal growth than any other pyrolysis product, we analyzed this correlation in more detail. When correlation between hyphal growth and TAME was studied separately for different organic amendments, the strongest correlation was observed for soil samples amended with cellulose (R=0.9103, Fig. 4). Weaker but still significant correlation was observed for soil samples without amendment. No significant correlation of TAME and hyphal growth was found in samples amended with root or shoot tissues (Fig. 4). The abundance of TAME tended to decrease with increasing time of incubation, indicating that organic compounds producing TAME during pyrolysis were probably decomposed by soil microorganisms (Fig. 4).

Table 2 Numbers of observed probable pyrolysis products of polysaccharides/light humus, various nitrogen-containing compounds (proteins), lignin, or humic substances and fatty acids showing significant correlation ($P \leq 0.05$, n=48) with fungal or plant growth

Parameter	Type of correlation	Polysaccharides	Nitrogen containing compounds	Lignin, humus	Fatty acids	Uncertain origin	Total
HG	+	3	1	11	4	14	33
	–	0	1	0	0	0	1
RLI	+	1	0	5	1	4	11
	–	1	0	0	0	0	1
RL	+	1	0	3	1	5	10
	–	1	0	0	0	0	1
Shoots	+	1	0	1	1	2	5
	–	0	0	0	0	0	0
All products		16	6	23	10	211	266

Compounds which were not identified or their origin cannot be deduced from their chemical nature are referred to as of "uncertain origin." Parameters describing fungal growth or plant growth are: growth of *G. intraradices* hyphae in vitro (HG), maize root length colonized by *G. claroideum* (RLI), total maize root length (RL), and maize shoot dry weight (Shoots) produced in hydroponic culture

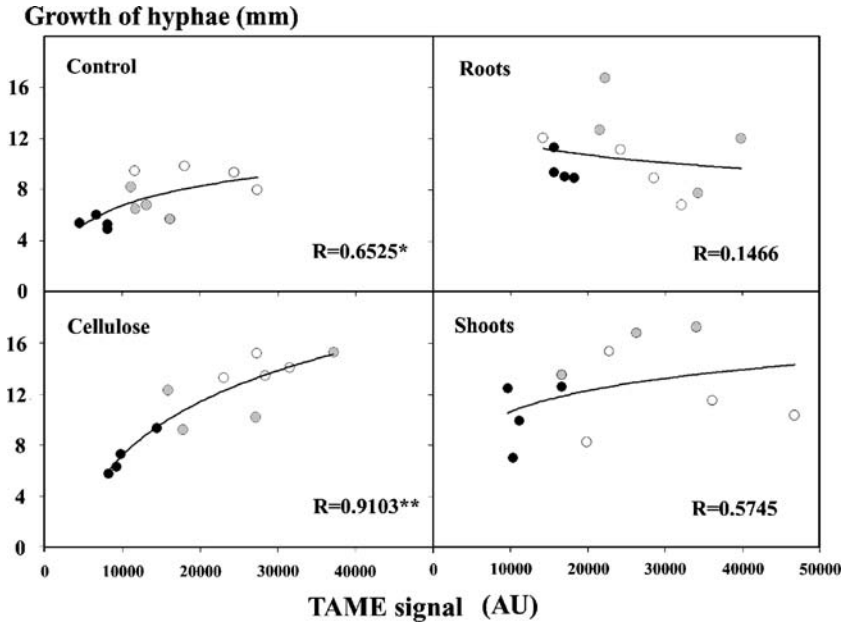

Fig. 4 Correlation of 3,4,5 trimethoxybenzoic acid methyl ester (TAME) signal with the growth of hyphae of *G. intraradices* in vitro. Content of TAME in volatilized products of soil pyrolysis is presented as a response of mass detector in arbitrary units (AU). Data obtained using soil samples incubated for 30 days (*white circles*), 100 days (*gray circles*), and 300 days (*black circles*) are presented separately for different types of organic amendments used

Some pyrolysis products tended to correlate positively with root length, colonized root length, and shoot dry weight of hydroponically grown maize, and only one product of pyrolysis of polysaccharides correlated negatively with root length and colonized root length (Tables 2 and 3).

Signal of TAME, summed over all measured samples, constituted 1.63% of summed signals of all the pyrolysis products. Nine other pyrolysis products gave stronger summed signals. Among them, the most abundant one was methoxyacetic acid methyl ester (code 33), which constituted 4.91% of summed signals of all pyrolysis products, followed by an unidentified compound constituting 3.52% of the summed signals. Neither of these two most abundant compounds showed significant correlation with hyphal growth, root colonization, or maize growth parameters. The third most abundant pyrolysis product constituting 2.80% of all summed signals was also unidentified and significantly positively correlated with growth of hyphae (R=0.3959). 1,2,4-Trimethoxybenzene (code 24), the fourth most abundant product (2.76% of all summed signals), positively correlated with hyphal growth (R=0.3919). Among other abundant pyrolysis products, an unidentified aliphatic hydrocarbon (code 38) constituting 1.82% of all summed signals showed significant positive correlation with hyphal growth (R=0.5149).

For the purposes of RDA, the number of explaining variables (at least partially identified pyrolysis products) had to be decreased since the method requires relatively low degree of correlation between explaining parameters. We thus excluded pyrolysis products 3-phenyl-2-propenoic acid methyl ester, 1,4-dimethoxybenzene and a methylated hexose from the analysis, since these compounds did not correlate with any of the explained parameters and were colinear with other pyrolysis products. We further summed the explaining parameters strongly positively correlating with TAME and analyzed them as one parameter (codes 2, 5, 6, 8, 9, 10, 11, 12, 14, 19, 20, 21, 23, 24, 26, 28, 35, 36, 37, 38, and 39). These actions minimized colinearity to allowable level. We further excluded all fatty acid esters produced during pyrolysis from the analysis. Fatty acids, as indicators of the presence of soil microorganisms, were analyzed directly and are presented as explained variables.

The results of RDA shown in Table 4 indicate that first canonical axis explains the largest portion of data variability. A total of 78.3% of variance in relations between explaining and explained parameters is explained by the first four canonical axes. Monte Carlo permutation test confirmed the significance of relationship between explaining and explained parameters for first axis as well as for all axes together.

Biplot presentation of RDA results is shown in Fig. 5. Codes denoting the chemical identity are given in Table 3. In Fig. 5, a close correlation of hyphal growth with pyrolysis products correlating with TAME (denoted as "corr25" in Fig. 5) can be observed. TAME (code 25), being the most closely associated with this parameter, is presented separately.

Plant growth parameters showed weaker correlation with pyrolysis products (TAME) than hyphal growth. Absorbance of the soil extract at 280 nm tended to be weakly related to the same products as hyphal growth. pH of the soil extract tended to be negatively related to all the aforementioned parameters.

Relationship between TAME and WCFA

Seven different WCFA were observed to correlate positively (at $P \leq 0.01$, $n=48$) with TAME abundance in soil pyrolysis products: 10:0-3OH ($R=0.4483$), 15:0-iso ($R=0.6203$), 15:0-anteiso ($R=0.5528$), 16:0-iso ($R=0.4617$), 16:1ω7 ($R=0.5899$), 18:1ω7 ($R=0.4158$), and 20:4ω6,9,12,15 ($R=0.4122$).

Summed signals of these seven WCFA over all measured samples represented 24.1% of summed signals of all WCFA. In total, 35 different WCFA were analyzed. Among them, 18:1ω9 and 16:0 gave strongest signals (15.9% and 14.6% of all summed signals, respectively). Abundances of these two WCFA did not correlate with TAME.

As seen on RDA biplot (Fig. 5), WCFA were mostly looser associated with hyphal growth than majority of pyrolysis products.

Discussion

The results obtained from this experiment confirm our previous observation (Gryndler et al. 2002) showing that relatively low amounts of organic matter applied to soil can affect both plant growth (in terms of shoot dry weight or root length) and development of AM fungi, measured as colonized root length and hyphal growth.

In the hydroponic system, testing the ability of *G. claroideum* to colonize maize roots, plant growth seemed to be mainly influenced by the mineral nutrients released after decomposition of the applied alfalfa tissues. This is

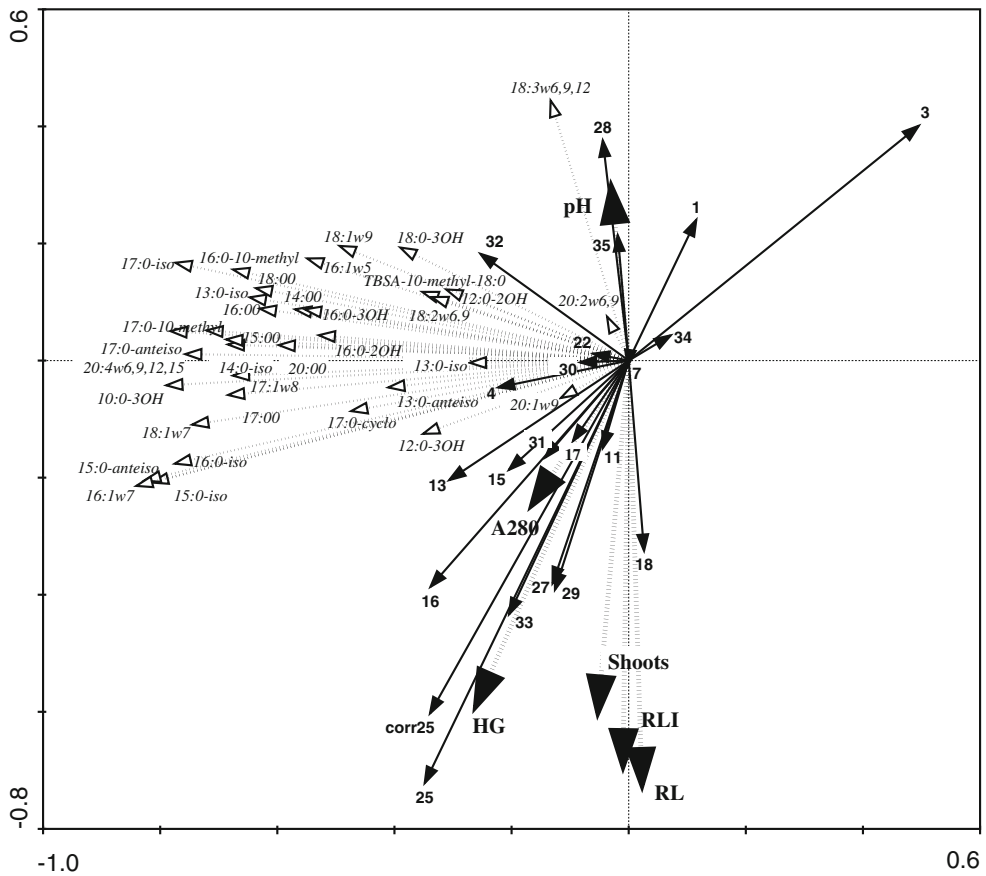

Fig. 5 Biplot presentation of relationships between pyrolysis products of soil organic matter (*arrows*) as explaining parameters and signature fatty acids (measures of abundance of soil microorganisms, *small white triangles*) and other measured explained parameters (*large black triangles*) as explained parameters. Marks "A280" and "pH" mean absorbance of the soil extract at 280 nm and its pH. Other marks as in Table 3. Data were analyzed by redundancy analysis and first two canonical axes are displayed. Scores of pyrolysis products (*arrows*) are marked by codes explained in Table 3. The mark *corr25* denotes the score of pyrolysis products closely correlating with TAME. For clarity, TAME itself is presented separately (*arrow with code 25*)

Table 3 Correlations between signal intensities of chemical species identified or partially characterized among derivatized pyrolysis products of soils and the growth of hyphae of *G. intraradices* (HG) exposed to soil extract in vitro, the length of maize roots colonized with *G. claroideum* (RLI), total root length (RL) and shoot dry weight (Shoots) of maize plants

	HG	RLI	RL	Shoots	Code
N-containing compounds (proteins)					
2-Acetamino-5-methylimidazole	−0.2385	−0.1733	−0.1699	−0.1580	1
1,3-Dimethyl-2,4(1H,3H)-pyrimidinedione	0.1263	0.1625	0.1408	0.1690	2
3-Methoxy-4-nitro-3-butenoic acid ME[a]	**−0.3952**	−0.2710	−0.2587	−0.2328	3
?-Methyl-1H-indole	0.0622	0.0114	−0.0135	−0.0401	4
?-Methyl-5-oxo-proline ME	**0.4758**	0.3441	0.3248	0.3196	5
Unidentified N-containing compound	0.3342	0.2229	0.2596	0.2730	6
Humus/lignin					
?,?-Dihydroxybenzoic acid	−0.0281	−0.0555	−0.0796	−0.1027	7
3,4-Dimethoxybenzaldehyde	0.3337	**0.3894**	**0.3757**	0.2838	8
3,5-Dimethoxybenzaldehyde	**0.4441**	**0.4134**	0.3688	0.2452	9
1,2-Dimethoxybenzene	**0.4189**	0.1857	0.1406	0.1105	10
1,3-Dimethoxybenzene	−0.1060	0.0880	0.0802	0.0843	11
1,4-Dimethoxybenzene	0.3412	0.1796	0,1518	0.1351	–
3,4,-Dimethoxybenzoic acid ME	**0.4847**	**0.4166**	**0.4046**	0.3626	12
3,5-Dimethoxyphenol	**0.4132**	0.0089	−0.0383	−0.0398	13
1-(3,4-Dimethoxyphenol)-ethanone	**0.4554**	0.2535	0.2314	0.0649	14
2,6-Dimethoxy-4-(2-propenyl)-phenol	0.0822	0.1439	0.1497	0.1442	15
4-Ethenyl-1,2-dimethoxybenzene	0.2840	0.2082	0.2043	0.1304	16
4-Ethyl-2-methoxyphenol	0.1203	0.0211	0.0101	−0.0211	17
4-Methoxybenzaldehyde	0.1224	0.1354	0.1437	0.1566	18
4-Methoxybenzoic acid ME	**0.4371**	0.2862	0.2746	0.2654	19
?-Methoxybenzoic acid ME	**0.3842**	**0.3751**	0.3675	0.3702	20
3-(4-Methoxyphenyl)-2-propenoic acid ME	0.2850	0.3071	0.2791	0.2665	21
3-Phenyl-2-propenoic acid ME	0.0001	−0.0486	−0.0526	−0.0460	–
?,?,?,?-Tetramethoxybenzene	−0.0513	0.0005	0.0121	0.0311	22
1,2,3-Trimethoxybenzene	**0.4336**	0.0842	0.0382	0.0277	23
1,2,4-Trimethoxybenzene	**0.3919**	0.1416	0.1145	0.0842	24
3,4,5-Trimethoxybenzoic acid ME	**0.5431**	**0.4438**	**0.4240**	**0.3909**	25
1-(3,4,5-Trimethoxyphenol)-ethanone	**0.4900**	0.3378	0.3561	0.3165	26
Unidentified aromatic structure	0.1985	0.2493	0.2413	0.2170	27
Fatty acids					
10-Methylundecanoic acid ME	0.1554	0.2515	0.1941	0.1403	–
Nonanediocic acid dME[b]	**0.4032**	0.3038	0.2804	0.2546	–
12-Methyltridecanoic acid ME	0.3197	0.3149	0.2719	0.2185	–
Tetradecanoic acid ME	0.1809	0.2732	0.2258	0.1615	–
?-Methyltetradecanoic acid ME	**0.4627**	0.2757	0.2359	0.1733	–
Pentadecanoic acid ME	0.2187	0.2803	0.2478	0.1874	–
14-Methylpentadecanoic acid ME	0.3613	0.2379	0.2207	0.1710	–
?(9?)-Hexadecenoic acid ME	**0.5318**	**0.4445**	**0.4148**	**0.3750**	–
?-Hexadecenoic acid ME	0.1187	0.0319	−0.0538	−0.2213	–
Hexadecanoic acid ME	**0.4157**	0.3274	0.2855	0.2173	–
Polysaccharides /light humus					
3,4-Dihydro-4-hydroxy-2H-pyran	−0.1326	**−0.4241**	**−0.3816**	−0.1984	28
1,1-Dimethoxyhexane	0.3226	0.3666	0.3241	0.2241	29
1,1-Dimethyl-1-propanol	0.1302	0.0357	−0.0610	−0.0592	30
2-Formylfuran	0.2845	0.0750	−0.0906	−0.0278	31
2,4-Heptadienoic acid ME	0.0486	−0.1933	−0.2433	−0.1825	32
Methoxyacetic acid ME	0.3596	0.2177	0.1812	0.1930	33
Methylated hexose	−0.1258	−0.1457	−0.1521	−0.1254	–
3-Methylbutanoic acid ME	0.0090	−0.0771	−0.0349	0.0250	34
5-Methyl-2-furancarboxaldehyde	0.0655	−0.2417	−0.2903	−0.2441	35
Unidentified aliphatic hydrocarbon	**0.4651**	0.3078	0.3023	0.2703	36
Unidentified aliphatic hydrocarbon	**0.5149**	0.2189	0.1788	0.1681	37

Table 3 (continued)

	HG	RLI	RL	Shoots	Code
Unidentified aliphatic hydrocarbon	**0.4312**	0.1883	0.1602	0.1474	38
Unidentified aliphatic hydrocarbon	0.3420	**0.4638**	**0.4712**	**0.5620**	39

[a] *ME* methyl ester
[b] *dME* dimethyl ester
Significant correlation coefficients ($|R|>0.372$, $P=0.01$) are given in bold letters. The rightmost column contains codes denoting the positions of product scores in Fig. 5.

supported by the increase in plant growth when mineral nutrients were applied as N-, P-, and K-containing salts. The concentration of phosphorus in hydroponic nutrient solution was relatively low, so that the accessibility of this element was probably limiting the growth of maize test plants and a small amount of phosphorus introduced into the hydroponic substrate as a component of applied plant tissues (Ha et al. 2007) might have affected plant growth. The root length colonized by *G. claroideum* behaved similarly as other plant growth parameters, which were enhanced by increased mineral nutrition. This result agrees with observations of Bethlenfalvay et al. (1982) and Kelly et al. (2005) who found decreased mycorrhizal colonization and root weight colonized, respectively, at very low phosphorus availability.

In vitro hyphal growth of *G. intraradices* behaved differently than root colonization by *G. claroideum*, being driven mainly by organic amendment and independent of mineral N and P availability. This suggests that the growth of mycelium of AM fungi is sensitive to the soluble fraction of soil organic matter, which agrees with our previous observations (Gryndler et al. 1998).

In general, pyrolysis with in situ methylation enables an insight of the soil organic matter as a whole, including insoluble components. This method has successfully been used in analyses of different forms of soil organic matter (e.g., Dai et al. 2002; Hermosin et al. 2001; Olk et al. 2002). More recently, it was used in identification of fungi on the basis of mycelium-specific pyrolysis products (Schwarzinger 2005). The method has also been used in studies of wood decomposition by fungi (Vane 2003; Vane et al. 2003) and is thus applicable for analyses of microbial transformation of complex organic compounds as employed in the present study.

Our data revealed that the *G. intraradices* hyphal growth responded to specific organic matter components, which released soluble traces into the soil solution. Among these products, TAME was recognized as most closely correlating with hyphal growth of the AM fungus.

The signal strength produced by pyrolysis GC-MS may be taken as a rough measure of relative amounts of pyrolysis products in the analyzed sample. Assuming this, it can be accepted that TAME or the second most abundant compound positively correlating with hyphal growth, 1,2,4-trimethoxybenzene, are not the major components present in soil pyrolysis products. Their signals are much weaker than the signal of methoxyacetic acid methyl ester that does not show significant correlation with hyphal growth of *G. intraradices*. Furthermore, hyphal growth correlated with TAME more closely than with overall extracted organic compounds detectable in soil extract at 280 nm. These facts indicate that increase in hyphal growth of the mycorrhizal fungus was not associated with soil organic matter in general but rather with a fraction enriched in structures chemically related to 3,4,5-trihydroxybenzoic acid or syringic acid, which were converted to TAME during pyrolysis (Simonelt et al. 1993).

A question appears about the origin of these structures in our samples. They are characteristic for lignin formed in angiosperm woody plants, and their derivatives are present in wood pyrolysis products (Simonelt et al. 1993). Humus formed from the litter of these angiosperms should be

Table 4 Results of redundancy analysis (RDA) of interaction between at least partially identified products of pyrolysis of soil organic matter except fatty acid methyl esters (explaining variables) and parameters describing directly measured WCFAs, growth of AM fungal growth, plant mycorrhizal colonization and plant growth (explained variables)

	Total variability			
Axes	1	2	3	4
Eigenvalues	0.310	0.062	0.048	0.033
Cumulative percent variance				
of explaining parameters	31.0	37.2	42.0	45.3
of explaining-explained relation	53.5	64.2	72.5	78.3
Monte Carlo permutation test				
First canonical axis significance				
Eigenvalue	0.310			
F ratio	11.223			
P value	0.0380*			
All canonical axes significance				
Trace	0.579			
F ratio	1.564			
P value	0.0460*			

enriched mainly in syringyl structures. However, the increased abundance of TAME in products of soil pyrolysis caused by addition of pure cellulose clearly indicates that these structures are of microbial origin, which may be formed in soil independently of the input of woody litter.

Soil microorganisms, including fungi, possess the shikimate pathway producing various aromatic compounds, including compounds containing 3,4,5-hydroxylated benzyl group (Turner 1971), a precursor of many aromatic microbial metabolites including 3,4,5-trihydroxybenzoic acid and its substituted derivatives.

Interestingly, the strength of correlation between TAME detection and hyphal growth depended on the type of organic matter applied to the soil. Unlike the soil treated with cellulose or control soil, the soil samples receiving root or shoot tissues did not show correlation between TAME and hyphal growth. Cellulose may be quickly colonized by certain fungi, which may convert it to humus-like aromatic structures (Capoor et al. 1978; Mishra et al. 1979), forming humus that increases the growth of AM fungi (Gryndler et al. 2005). Plant tissues, i.e., roots or shoots, are a more complex substrate for decomposition and may be colonized by different microorganisms. This may result in higher data variability than in the case of pure cellulose, so that the correlation coefficients remain insignificant.

The main groups of soil microorganisms potentially responsible for production of organic matter enriched in substituted derivatives of 3,4,5-trihydroxybenzoic acid may be inferred by using a correlation of characteristic fatty acids (denoting the presence of particular microbial groups) with TAME abundance in soil pyrolysis products. Fatty acids, which positively correlated with TAME in our experiment, are biomarkers of G+ bacteria (15:0-iso, 15:0-anteiso, 16:0-iso, Ratledge and Wilkinson 1988), G− bacteria (10:0-3OH, Wilkinson 1988), fungi and G− bacteria (16:1ω7, 18:1ω7, Ratledge and Wilkinson 1988), and protozoa (20:4ω6,9,12,15, Lechevalier and Lechevalier 1988). The lack of significant positive correlation of TAME with methylated WCFA known from actinomycetes (Wilkinson 1988) is surprising. It may indicate that syringyl structures did not accumulate significantly in the examined soil samples rich in actinomycetes.

Our observations suggest that fungi, bacteria, or protozoa may be important for the formation of 3,4,5-substituted benzyl structures in soil organic matter, and this may indirectly affect the growth of AM fungi. However, the correlation between certain microbial groups and TAME-containing organic matter may also simply indicate that these microorganisms are feeding on these compounds. The WCFA method employed in the present study is not sufficiently specific to allow precise conclusions about the role of different groups of soil microorganisms in accumulation of these structures in soil, and more versatile methods and experimental designs should be employed in future research in this field. However, the lack of correlation between TAME signal and the very abundant WCFA 18:1ω9 (found in G− bacteria and mainly in higher fungi, Karliński et al. 2007) and 16:0 (found in all organisms, Ratledge and Wilkinson 1988) may indicate that not all groups of soil microorganisms are eqally involved in the turnover of 3,4,5-substituted benzyl structures, and some specificity has to be considered.

Relatively small amounts of organic matter can affect AM fungal growth. Whereas AM fungal root colonization depended on host plant growth and mineral nutrient availability, asymbiotic AM hyphal growth was affected by the organic amendment rather than by mineral nutrients. This indicates that limited asymbiotic growth of AM fungi, which may be important for example in fast colonization of winter crops, can be driven by the presence of buried decomposing plant tissues, residuals after the preceding harvest.

In conclusion, the results from the present experiment support our main hypothesis that specific compounds released during organic matter decomposition are involved in the AM fungal asymbiotic growth response associated with organic matter amendment. Our results indicate that growth of mycelium of AM fungi is preferentially associated with a specific fraction of soil organic matter enriched in 3,4,5-substituted benzyl structures. Also, it seems that other soil microorganisms produced at least some of these compounds.

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