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Fermentation of sugar beet waste by *Aspergillus niger* facilitates growth and P uptake of external mycelium of mixed populations of arbuscular mycorrhizal fungi

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Abstract

Sugar beet waste has potential value as a soil amendment and this work studied whether fermentation of the waste by *Aspergillus niger* would influence the growth and P uptake of arbuscular mycorrhizal (AM) fungi. Plants were grown in compartmentalised growth units, each with a root compartment (RC) and two lateral root-free compartments (RFC). One RFC contained untreated soil while the other RFC contained soil, which was uniformly mixed with sugar beet waste, either untreated (SB) or degraded by *A. niger* (ASB) in a rock phosphate (RP)-supplied medium. The soil in each pair of RFC was labelled with ^{33}P and ^{32}P in order to measure P uptake by the AM fungal mycelium, of which length density was also measured. Whole cell fatty acid (WCFA) signatures were used as biomarkers of the AM fungal mycelium and other soil microorganisms. The amount of biomarkers of saprotrophic fungi and both Gram-positive and Gram-negative bacteria was higher in SB than in ASB treatments. Whilst ASB increased growth and activity of AM mycelium, SB had the opposite effect. Moreover, shoot P content was increased by the addition of ASB, and by inoculation with AM fungi. Modification of soil microbial structure and production of exudates by *A. niger,* as a consequence of fermentation process of sugar beet waste, could possibly explain the increase of AM growth in ASB treatments. On the other hand, the highest P uptake was a result of the solubilisation of rock phosphate by *A. niger* during the fermentation.

Keywords: Arbuscular mycorrhizal fungi; Sugar beet waste; *Aspergillus niger*; Biomarker fatty acids; Phosphorus uptake; Mycorrhiza hyphosphere

1. Introduction

Sugar beet waste is a lignocellulosic residue produced during sugar processing. This material is very cheap and is mainly used as animal feed. However, its use as an amendment has been recommended after microbial treatment with *Aspergillus niger* in a rock phosphate (RP)-amended medium (Rodriguez et al., 1999; Vassilev et al., 1996).

A. niger grown on SB waste secretes organic acids, citric acid in particular, and this fermentative process leads to solubilisation of RP and transformation of the lignocellulosic material into more simple sugar compounds (Vassilev et al., 1986, 1995). In order to assess the fertiliser value of this improved fermented waste it is important to examine its influence on plant beneficial microorganisms, such as arbuscular mycorrhizal (AM) fungi, which play a key role in soil fertility and in plant nutrient acquisition (Azcón-Aguilar and Barea, 1992; Requena et al., 1996). Plant growth promotion in response to *A. niger*-treated SB waste was accompanied by increased AM root colonisation (Vassilev et al., 1996; Rodriguez et al., 1999; Medina et al., 2004). However, it is unknown how fermented or unfermented SB waste affects the extraradical phase of AM fungi and the general soil microbiota.

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The main objective of the present study was to examine the influence of SB waste fermented by *A. niger* in RP-supplied medium (ASB) on growth and activity of external mycelium of AM fungi. The use of pots with two root-free soil compartments made it possible to study the influence of ASB on AM mycelium without interfering effects from roots. Also the influence of organic amendments and AM fungi, alone and in combination, on other soil microorganisms and plant nutrient content were measured.

2. Materials and methods

2.1. Experimental design and pots

Mycorrhizal and non-mycorrhizal plants were grown in compartmented pots (Larsen and Jakobsen, 1996), which were made from PVC tubes (4.5 cm internal diameter). Each pot consisted of a 32.5 cm long central root compartment (RC) and two 7 cm long lateral root-free compartments (RFC); sheets of 37 μm nylon mesh prevented root penetration, but allowed AM mycelium to grow into the RFCs (Fig. 1). RCs were sealed at the bottom by a coarse nylon mesh. Soil in one RFC of each pot referred to as ARFC (amended RFC) was mixed with fermented or unfermented SB waste at 50 mg g^{-1} soil and $H_3^{32}PO_4$ at 2.44 kBq g^{-1} soil. The soil of the other RFC referred to as UARFC (unamended RFC) was mixed with $H_3^{33}PO_4$ (3.37 kBq g^{-1} soil), but received no amendment. The isotopes were added as carrier-free solution. The main

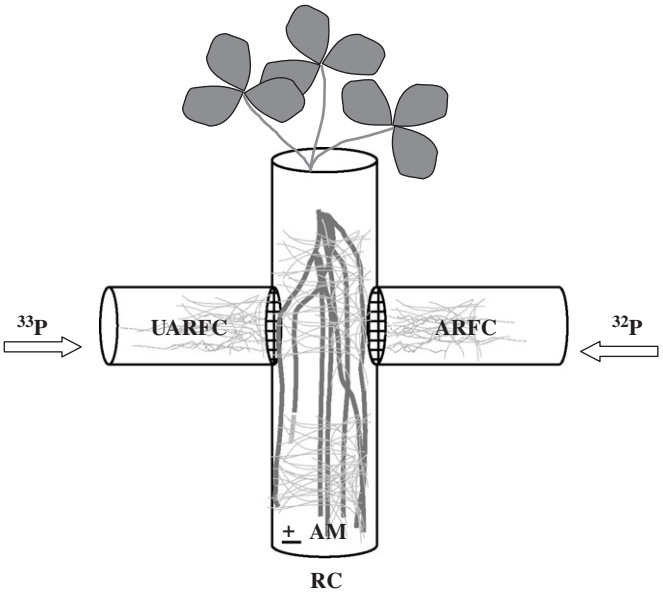

Fig. 1. Design of the compartmentalised growth system with a central root compartment (RC) and two lateral root-free compartments (RFCs) separated by 37 μm nylon mesh. One of the RFC, named ARFC, was amended with SB or ASB and only remained unamended in the control treatments with mycorrhiza. The other RFC was named UARFC and did not receive any amendment. Arrows indicate application of tracer isotopes. For more details see materials and methods.

treatments (±AM × two types of amendment) had five replicates with a total of 20 growth units. Five mycorrhizal pots with no amendment in both RFCs were included as P transport controls.

2.2. Fermentation process

Sugar beet waste was ground in 1 mm fragments and 5 g were mixed with 50 ml Czapek's solution (Fluka Chemica, catalogue no 70185) in 250 ml Erlenmeyer flasks. RP (Morocco fluorapatite, 12.8% soluble P, 1 mm mesh) was added at a rate of 0.75 g 50 ml^{-1}. The media was sterilised by autoclaving at 120 °C for 30 min. The mixture was inoculated with 3 ml of a spore suspension (1.2×10^6 spores) of *A. niger* strain NB2, which produces citric acid when grown on complex substrates (Vassilev et al., 1986). The fermentation was performed at 28 °C for 20 days under still conditions.

2.3. Soil, amendments, and inoculation with AM fungi

The soil was a 1:1 (w/w) mixture of a sandy loam and quartz sand, which had been irradiated (10 kGy, 10 MeV electron beam) to eradicate indigenous AM fungi. The soil mixture had a pH of 6.1 and contained 8 mg kg^{-1} soil of 0.5 M $NaHCO_3$-extractable P. The following nutrients were mixed into the soil (mg kg^{-1} soil): K_2SO_4 (70), $CaCl_2$ (70), $CuSO_4$ $5H_2O$ (2.2), $ZnSO_4$ $7H_2O$ (5), $MnSO_4$ $7H_2O$ (10), $CoSO_4$ H_2O (0.33), $NaMoO_4$ $2H_2O$ (0.2) and $MgSO_4$ $7H_2O$ (20).

The nutritional characteristics of the amendments [fermented (ASB) or unfermented (SB) sugar beet waste], were as follows (g kg^{-1}): N [12.5 (SB), 14 (ASB)]; P [1.5 (SB), 3.5 (ASB)], K [2.5 (SB), 3.2 (ASB)]. SB was sterilised (120 °C, 30 min) before being added to the ARFC.

Mycorrhizal plants were obtained by inoculation with a mixture of three AM fungi isolated from Riotinto (Spain), *Glomus intraradices* (EEZ-54), *G. mosseae* (EEZ-55) and *Acaulospora trappei* (EEZ-56). Each AM fungus was propagated in open pot cultures of *Trifolium repens* L. Inoculum for each pot contained 10 g soil from each pot culture, consisting of spores, mycelium and mycorrhizal root fragments.

The central RCs were filled with 730 g of soil. The treatments with AM consisted of a bottom layer of 350 g of soil, a middle layer of a mixture of 220 g soil and 30 g inoculum, and a top layer of 130 g of soil. In order to reintroduce microorganisms other than AM fungi, all growth units received 10 ml of a soil filtrate obtained from a suspension of 30 g inoculum in 500 ml H_2O, which had been sieved through a 20 μm nylon mesh.

2.4. Plants and growth conditions

Five seeds of *T. repens* L. were sown in each pot, which were thinned to two plants per pot after seedling emergence. Plants were maintained in a growth chamber

with a 16/8 h light/dark cycle at 21/16 °C. Osram daylight lamps (HQ1-T 250 W/D) provided a photosynthetically active radiation of 500–550 mmol m^{-2} s^{-1}. The plants were relocated to minimise variation and watered daily by weight to maintain 60% of the field capacity.

2.5. Harvest and analyses

Plants were harvested six weeks after sowing. Roots were washed free of soil and two subsamples were weighed, one of which was used for determination of root dry weight and the other was cleared in 10% KOH and stained in trypan blue by a modification of the method of Phillips and Hayman (1970), omitting phenol from the reagents and HCl from the rinse. Percentage of root system colonised was calculated by means of the line-intercept method (Newman, 1966). All other plant materials were dried, weighed and acid digested (Murphy and Riley, 1962). Total P and N content of shoots were measured using the molybdate blue and kjeldahl method, respectively, on a technicon autoanalyser II (Technicon autoanalysers; Analytical instrument recycle; Inc.; Golden co; USA). Potassium (K) was determined by flame photometry. Three ml of the diluted digest was mixed with 10 ml scintillation fluid, and ^{32}P and ^{33}P were counted on a Packard TR1900 liquid scintillation counter by dual spectrum analysis.

The two RFCs were removed from each growth unit and stored in the freezer at −18 °C until further analysis. Hyphal length densities in the UARFC and ARFC soil were measured by a grid-line-intersect method (Jakobsen et al., 1992). Whole cell fatty acids (WCFAs) were extracted from the soil samples (2.5 g) from both UARFC and ARFC by a four-step fatty acid methyl extraction procedure according to a method by Sasser (1990); saponification, methylation, extraction, and base wash. To enable quantification of the extracted fatty acid methyl esters an internal standard, a known amount of nonadeconoate fatty acid methyl ester 19:0, was added to each sample. Gas chromatography analyses and identification of the fatty acids was according to the software library TSBA41 (Parsley, 1996). Fatty acid 16:1ω5c was used for AM fungi (Olsson et al., 1998), 18:2ω6,9 for saprophytic fungi (Frostegård and Bååth, 1996; Larsen et al., 1998), branched chain localised on positions *iso* and *anteiso* (i14:0, i15:0, i16:0, i17:0, i19:0) for Gram-positive bacteria (Wilkinson, 1988; Zelles, 1997), cyclopropyl fatty acid (cy17:0, cy19:0) for Gram-negative bacteria (Wilkinson, 1988; Zelles, 1997), and 10 Me 16:0, 10 Me 17:0 and 10 Me 18:0 for actinomycete genera (Frostegård et al., 1992; Ravnskov et al., 2002).

2.6. Statistics

Levels of significance of the results from the main treatments and their interaction were obtained by analysis of variance and means were compared by $LSD_{0.05}$ multiple range test. Statgraphics Plus for Windows was used to perform the statistical test. Values for percentage root length colonised with AM fungi were ARC-sin transformed before statistical analysis. Total shoot P values were square root transformed before statistical analysis.

3. Results

3.1. Plant growth, nutrition and mycorrhiza formation

Plant shoots and roots from treatments both without and with AM developed similarly, except in the treatment without AM and with SB where shoot and root dry weights were reduced compared to the treatments with ASB (Table 1). All plants were nodulated despite that they were not inoculated with *Rhizobium*. Based on general visual inspection level of nodulation seemed to be similar in all treatments, but this was not quantified.

In AM-inoculated plants, the percentage of AM colonisation in roots was increased by 35% in the treatment with ASB and SB compared to AM plants without amendments (Table 1). Roots from pots without AM fungus inoculation remained mycorrhiza free.

The ASB amendment increased total shoot N and K contents in non-AM plants (Table 2). P acquisition increased in AM plants and they reached the highest value with ASB amendment (Table 2). Plant P content was 272% higher in AM plants treated with ASB compared non-AM plants treated with SB (Table 2).

3.2. AM-hyphal length and P transport

The hyphal length density in the UARFC in treatments without AM remained unaffected by SB and ASB with an

Table 1
Shoot and root dry weights and AM fungi root colonisation (%) as affected by inoculation with AM fungi in the root compartment and organic amendments in the ARFC

Mycorrhiza	Soil amendment		
	C	SB	ASB
Shoot dry weight (mg)			
AM	510±40 ab	428±91 ab	578±74 b
Non-AM		334±63 a	566±23 b
Root dry weight (mg)			
AM	164±30 ab	149±37 ab	218±26 bc
Non-AM		133±25 a	268±10 c
AM colonisation (%)			
AM	49±4 a	62±2 b	66±4 b
Non-AM		0*	0*

Values represent means ($n = 5 \pm$SE).
Values of all treatments of each parameter not sharing a letter in common differ significantly ($P < 0.05$ from each other) as determined by LSD multiple range test. Values followed by * were not included in LSD multiple range test.
C, unamended control; SB, sugar beet waste; ASB, *Aspergillus niger*-fermented sugar beet waste.

average density of 0.58 m g^{-1} soil. This background was subtracted from the values obtained in the corresponding treatments with AM. Whilst in treatments with AM the values of hyphal length densities in the soil of the UARFC were similar, with a mean of 4.3 m g^{-1} soil, in soil from ARFC hyphal length densities were differently affected by SB and ASB (Fig. 2a). Mycelia of AM fungi were not detected in treatments where SB was added to the ARFC. Nevertheless, hyphal length was 70% higher in soil from ARFCs with ASB in treatments with AM when compared to soil from the UARFCs from the corresponding treatments with AM, resulting in a significant positive interaction between ASB amendment and mycorrhiza (Fig. 2a).

In AM plants, there was no ^{32}P uptake when SB was added to the soil in the ARFCs, whereas the average ^{32}P content in plants where ASB was added to the soil in the ARFCs was 35 kBq (Table 3). Shoot ^{33}P content was on average 25 kBq and did not differ between the treatments (with SB or ASB and without amendments) in plants with AM (Table 3). ^{33}P and ^{32}P values of non-AM plants were considered residual. Length-specific uptake of ^{32}P from the ARFC in the treatment with SB was zero whereas that of ^{33}P from the UARFC was similar to length-specific uptake of ^{32}P and ^{33}P in the treatment with ASB, with a mean value of 5.81 kBq m^{-1} hypha.

3.3. Content of WCFAs in RFC

The concentration of WCFA 16:1ω5 in treatments without AM was low (0.018 nmol g of $soil^{-1}$) and unaffected by any treatment. After the corresponding background was subtracted, the amount of WCFA 16:1ω5 in the soil from the UARFC was not affected by the treatments in the ARFC (Fig. 2b). In contrast, in the ARFC from treatments with AM, WCFA 16:1ω5 was markedly influenced by the two different amendments. In

Table 2
Total shoot N, P, K content (mg) as affected by inoculation with AM fungi in the root compartment and organic amendments in the ARFC

Mycorrhiza	Soil amendment		
	C	SB	ASB
N content (mg)			
AM	4.15±1.09 ab	3.82±1.15 ab	5.34±0.29 b
Non-AM		3.06±0.02 a	5.82±0.28 b
P content (mg)			
AM	1.83±0.07 c	1.37±0.23 b	2.68±0.21 d
Non-AM		0.72±0.14 a	1.14±0.04 b
K content (mg)			
AM	0.80±0.16 ab	0.82±0.23 ab	1.21±0.05 b
Non-AM		0.67±0.30 a	1.21±0.07 b

Values represent means ($n = 5 \pm$SE).
Values of all treatments of each parameter not sharing a letter in common differ significantly ($P < 0.05$ from each other) as determined by LSD multiple range test.
C, unamended control; SB, sugar beet waste; ASB, *Aspergillus niger*-fermented sugar beet waste.

Table 3
Shoot ^{33}P and ^{32}P content (kBq) as affected by inoculation with AMF in the root compartment and organic amendments in the ARFC

Mycorrhiza	Soil amendment		
	C	SB	ASB
^{33}P *content* (kBq)			
AM	29.15±1.91 a	24.38±6.16 a	28.02±2.44 a
Non-AM		1.44±0.27 x	2.13±0.73 x
^{32}P *content* (kBq)			
AM	30.40±1.43 a	0.03±0.03 *	35.18±2.67 a
Non-AM		0.15±0.05 y	1.38±0.22 z

Values represent means ($n = 5 \pm$SE).
LSD multiple range test of shoot ^{32}P and ^{33}P content has been determined separately for AM and non-AM plants; values not sharing a letter in common differ significantly ($P < 0.05$ from each other). Values followed by * were not included in LSD multiple range test.
C, unamended control; SB, sugar beet waste; ASB, *Aspergillus niger*-fermented sugar beet waste.

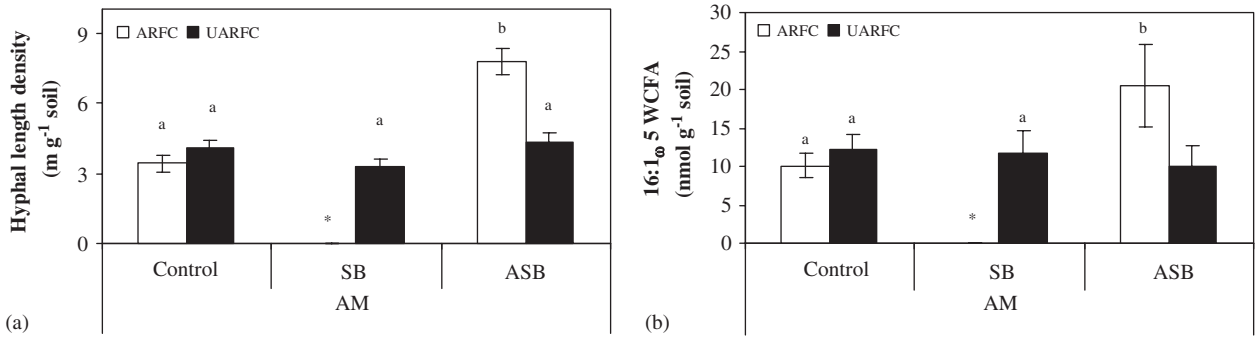

Fig. 2. Hyphal length densities (a) and concentration of WCFA biomarker of AM fungi (b) in soil from root-free compartments as affected by inoculation with AMF in the root compartment and soil amendments in the ARFC (C (unamended control), SB (sugar beet waste) and ASB (*Aspergillus niger* fermented sugar beet waste)). Vertical bars represent standard errors, $n = 5$. Values not sharing a letter in common differ significantly ($P < 0.05$ from each other) as determined by LSD multiple range test. Values followed by * are not included in LSD multiple range test.

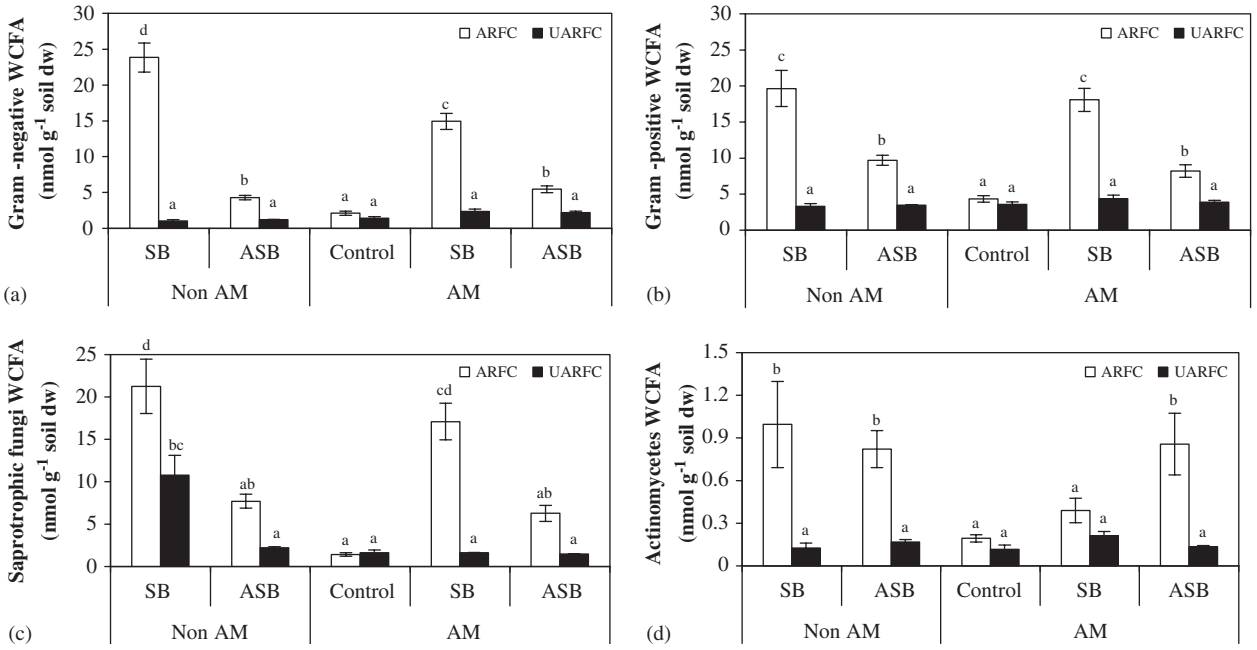

Fig. 3. Concentration of WCFA biomarkers Gram-negative bacteria (a), Gram-positive bacteria (b), saprotrophic fungi (c), and *Actinomycetes* (d) as affected by inoculation with AM fungi in the root compartment and soil amendments in the ARFC (C (unamended control), SB (sugar beet waste) and ASB (*Aspergillus niger* fermented sugar beet waste)). Vertical bars represent standard errors, $n = 5$. Values not sharing a letter in common differ significantly ($P < 0.05$ from each other) as determined by LSD multiple range test.

the ARFC with SB, 16:1ω5 was not detected, whereas the amount of 16:1ω5 content was 119% higher in the ARFC with ASB than in the corresponding UARFC (Fig. 2b).

In soil from the UARFC, the amount of WCFA biomarkers of Gram-negative bacteria was similar in all treatments (Fig. 3a). In soil from ARFC amended with SB, the amount of WCFA biomarkers of Gram-negative bacteria was 456% and 174% higher than that of soil from ARFC with ASB in treatments without and with AM, respectively (Fig. 3a). There was a significant interaction between amendment and mycorrhiza. The amount of WCFA biomarkers of Gram-negative bacteria in soil from the ARFC amended with SB was 37% lower in treatments with AM than without AM (Fig. 3a).

The addition of SB and ASB amendments to ARFCs increased WCFA biomarkers of Gram-positive bacteria up to 400% and 145%, respectively, compared to that of the unamended controls (Fig. 3b). Treatments with AM were not significantly different from their corresponding treatments without AM.

The amount of WCFA 18:2ω6,9 (biomarker of saprotrophic fungi) in soil from the UARFC were similar in all treatments, except in the treatment without AM with SB in the ARFC, which was 507% higher than in the rest of treatments of UARFC (Fig. 3c). The addition of the amendment SB to the ARFCs increased the amount of 18:2ω6,9 in the soil from the UARFC. Inoculation with AM had no effect on the amount of WCFA 18:2ω6,9 in soil from the ARFCs irrespective of the amendments (Fig. 3c). Biomarker WCFAs of *Actinomycetes* (methylated fatty acids) in soil from UARFC did not differ between treatments (Fig. 3d). Addition of the amendments to the soil in ARFC of both treatments without and with AM increased the amount of *Actinomycetes* WCFA biomarkers compared to that in the corresponding UARFC, except in the treatment with AM and SB where the amount of *Actinomycetes* was similar in both RFCs (Fig. 3d). The amount of *Actinomycetes* WCFA biomarkers in soil from ARFC was similar in treatments with SB and ASB in non-AM plants (Fig. 3d). In contrast, in AM plants the amount of *Actinomycetes* WCFA biomarkers in the soil of the ARFC amended with SB, was 55% lower than that of ASB (Fig. 3d).

The results of factorial ANOVA for plant and microorganisms parameters are given in Tables 4 and 5, respectively.

4. Discussion

4.1. Plant growth and nutrition

The higher shoot N, P and K content in non-AM plants from the treatment with ASB, compared to the treatment with SB could be due to the mineralisation of the waste and the solubilisation of RP that takes place during the fermentation process of SB waste by *A. niger* (Vassilev et al., 1995, 1996). The diffusion of K and N from the amended compartment to the RC could explain the similar content of these two nutrients in AM and non-AM plants from treatments with ASB.

Table 4
Mean squares values followed by level of significance from two-way analyses of variance for the plant parameters measured with three levels of amendment in the ARFC (none, sugar beet waste and *Aspergillus niger* fermented sugar beet waste) and two levels of mycorrhiza (without and with)

Plant parameters	Amendment (A)	Mycorrhiza	(M) AxM
P total content	641.1130 ***	985.9050 ***	73.3396 ns
^{33}P (kBq)	2.3484 ns	2.9789 ***	1.0914 ns
^{32}P (kBq)	1.6550 ***	1.4184 ***	1.4376 ***
K total content	1.0786 **	0.0282 ns	0.0322 ns
N total content	15.8675 *	0.0695 ns	1.3167 ns
Shoot dry weight	0.1824 **	0.0140 ns	0.0084 ns
Root dry weight	0.0520 **	0.0016 ns	0.0051 ns

Table 5
Mean squares values followed by level of significance from two-way analyses of variance for the parameters of microorganisms measured in the amended root-free compartment (ARFC) and the unamended root-free compartment (UARFC) with three levels of amendment in the ARFC (none, sugar beet waste and *Aspergillus niger* fermented sugar beet waste) and two levels of mycorrhiza (without and with)

Microbial parameters	Amendment(A)	Mycorrhiza (M)	AxM
Gram negative			
ARFC	0.5621 ***	0.0402 **	0.0682 ***
UARFC	0.0001 ns	0.0052 ***	0.0004 ns
Gram positive			
ARFC	0.2615 ***	0.0059 ns	0.0000 ns
UARFC	0.0001 ns	0.0017 *	0.0007 ns
Actinomycetes			
ARFC	0.0001 ns	0.0002 ns	0.0003 ns
UARFC	0.0000 ns	0.0000 ns	0.0000 ns
Saprotrophic fungi			
ARFC	0.3969 ***	0.0212 ns	0.0050 ns
UARFC	0.0510 ns	0.0655 ns	0.0471 ns

The low values of shoot dry weight in treatments without AM and with SB compared to the treatment with ASB could be explained by the diffusion of plant toxic components of SB waste from the amended root free compartment to the RC. Vassilev et al. (1996) also found a negative influence on *T. repens* growth when untreated SB waste was applied to the soil. Besides, a water extract of SB waste inhibited AM hyphal growth when added to the HC of monoxenic cultures (A. Medina, unpublished data).

4.2. AM hyphal growth

Unfermented sugar beet waste clearly inhibited the growth and activity of AM mycelium as indicated by measurements of hyphal length density, WCFA 16:1ω5 and ^{32}P uptake. These variables had values similar to those observed in treatments without AM. In contrast, the amount of the biomarker fatty acid for saprotrophic fungi (18:2ω6,9) was higher in soil with SB waste.

Hemicellulose and cellulose are the main constituents of SB waste, and in less quantity, lignine. Ravnskov et al. (1999) found that cellulose increased the biomass of saprotrophic fungi and suggested that these fungi could have adverse effects on AM fungal growth via antibiosis or competition for inorganic nutrients. McAllister et al. (1994) also reported that the accumulation of toxic substances produced by saprotrophic fungi could inhibit the growth of AM fungi. In contrast, studies designed to study interactions between AM fungi and saprotrophic fungi in RFCs showed that the AM fungus *G. intraradices* was unaffected by the presence of the saprotrophic fungi, *Trichoderma harzanium* (Green et al., 1999) and *Fusarium culmorum* (Larsen et al., 1998). However, these two studies only included specific saprotrophic fungi and not communities.

The increased AM fungal growth with ASB amendment is well supported by several authors that have shown similar enhanced growth of AM fungi in soil with organic matter (Hepper and Warner, 1983; St John et al., 1983; Joner and Jakobsen, 1995), but inhibitory effects of organic matter have been observed as well (Avio and Giovannetti, 1988; Calvet et al., 1992). Ravnskov et al. (1999) concluded that effects of organic compounds on growth of AM fungi in soil vary according to the chemical composition of the substrate. On the other hand, exudates secreted by *A. niger* has been shown to increase germination of spores of AM fungi and subsequent pre-symbiotic mycelial growth (Fracchia et al., 2004). In this way, the increased AM hyphal growth in soil with ASB amendment could be explained by the presence of these different compounds released during the fermentation, that were not present in the unfermented SB waste.

4.3. AM hyphal P transport

As mentioned earlier ASB amendment increased AM fungal growth, but this increase in growth did not result in a similar increase in AM hyphal P uptake as revealed from the ^{32}P uptake results. The fact that total shoot P in AM plants from treatments with ASB was 46% higher than in AM control plants, whereas ^{32}P uptake was similar on both

treatments, suggests that the main reason for the high P/^{32}P values in shoots of plants from the ASB treatment was not caused by the increase in AM hyphal length. Vassilev et al. (1996) demonstrated the ability of *A. niger* to solubilise RP when growing on SB waste medium and they observed an increase in total P content in shoot when this amendment was added to a soil plant system. Vassilev et al. (1996) suggested that citrate ions secreted by *A. niger* can be adsorbed at the same sites as phosphate in the soil system and consequently, may release phosphate ions directly maintaining P availability. In our experiment, the treated SB waste amendment may have provided a pool of available phosphorus to the ARFC as a consequence of RP solubilisation. This P was translocated by AM mycelium to the host plant resulting in a higher P/^{32}P ratio in shoots of AM plants from the treatment with ASB. On the other hand, a partial consume of the soluble P by *A. niger* reported by Vassilev et al. (1995), could explain that AM hyphal length specific uptake of ^{32}P was not more efficient than ^{33}P in ASB treatment, which indicates that ^{32}P uptake did not increase proportionally to AM hyphal length in ARFC with ASB. Green et al. (1999) also attributed the lack of correspondence between external mycelium growth of *G. intraradices* in response to wheat bran amendment and AM-mediated ^{33}P uptake to immobilisation by saprotrophic microorganisms, which also had increased growth with the organic amendment. Our results suggest that although there was a small consume of P, its availability in soil was still high enough to ensure a high P uptake by AM fungi.

4.4. Biomarker fatty acids of other soil microorganisms

The addition of organic matter to soil may alter soil microbial community structure and function (Zhao et al., 2005). In our experiment, as a general tendency, both SB and ASB amendments enhanced the amount of biomarker fatty acids of all groups of microorganisms, as a result of the increase of carbon source. Since SB waste is an untreated material, the amount of WCFA microbial biomarkers were higher than that of ASB, which is a partially biotransformed amendment.

Both positive and negative interactions between AM fungi and the surrounding microbiota have been reported (Secilia and Bagyaraj, 1987; Azcón-Aguilar and Barea, 1992; Christensen and Jakobsen, 1993). Microbial activity is of great importance in nutrient cycling and energy flow (Díaz Raviña et al., 1992), and also in producing beneficial substances for AM, but on the other hand, competition for metabolites between soil microorganisms and AM fungi may decrease the effectivity of the latter (Garcia-Romera et al., 1998). Therefore, the interaction between AM fungi and the surrounding microbiota depends on the balance of beneficial and detrimental factors. In our experiment, the increase in WCFA biomarkers of saprotrophic fungi, Gram-positive and Gram-negative bacteria in ARFCs was higher in SB than in ASB treatments. The different microbial structure in soil of both treatments could probably explain the opposite effects on AM and plant growth of SB and ASB substrates. The microbial community in the ARFC with ASB was unaffected by inoculation with AM fungi, but it seems that this microbial community structure created a favourable environment for the growth of AM fungi. On the other hand, several studies have shown that rhizosphere bacteria are affected by the presence of AM, and negative as well as positive effects of AM on bacteria have been reported. We found that AM fungi did not influence microbial populations in ARFC with ASB. However, it is important to note that WCFA biomarkers were measured in the hyphosphere, in absence of the influence of roots. The presence of roots would probably affect soil microbial structure. Indeed, Olsson et al. (1996) found no significant effect of the growing AM mycelium on bacterial community structure, whereas presence of mycorrhizal roots had a stimulatory effect.

5. General conclusions

We conclude that fermentation of SB waste by *A. niger* is crucial for its use as a soil fertiliser: the fermentation is beneficial to growth of AM fungi, increases the amount of available P and removes potential phytotoxic effects of the SB waste. The reason behind the ASB-induced increase in growth of the AM fungal mycelium could not be determined in this study, but our results suggest that modification of the structure of soil microbial communities, production of exudates by *A. niger* and changes in levels of available nutrients could have been involved.

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