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ORIGINAL PAPER

Improvement of soil fertility and enzymatic activity by wastewater sludge compost and arbuscular mycorrhizal fungi in giant reed's rhizosphere

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Abstract

The effect of low-dose, commercially available wastewater sludge compost (WSC; 15 t ha^{-1}) treatment was examined with or without arbuscular mycorrhizal fungal (AMF) inoculation on the nutritional status, heavy metal (HM) concentration and the rhizosphere activity of giant reed (*Arundo donax* L. var. BL clone (Blossom)) plants. *Funneliformis mosseae* (BEG12; AMF1), *F. geosporum* (BEG11; AMF2) or their combination (AMFmix) were applied as AMF treatments in a short-term pot experiment. The physiological and growth parameters of the host plants, the AMF root colonization and the microbiological enzyme activity of the mycorrhizosphere were examined. We assumed that the combined treatment (WSC + AMF) enhances the fertility of low-fertility acidic sandy soil. Neither the WSC treatment nor the AMF inoculations changed the extent of root colonization. Based on the results of root electrical capacitance and the phosphorous uptake, plant nutritional status was improved by WSC addition, without any negative impacts among the measured parameters. AMF treatments increased the enzyme activity in the soil and decreased the concentrations of the potentially toxic HMs (Cu, Mn, Pb, Zn) in roots, but that mitigation of Cu and Zn was compensated in shoots. According to the results of MicroResp™ measurements, the catabolic activity profile of the soil microbial community was changed in case of the AMF2 treatment. The efficient regulatory mechanism of giant reed might be able to adjust optimal/maximal colonization rate, and to select the preferential AMF partners, this supposed mechanism might be responsible for its invasiveness and tolerance to a wide range of environmental conditions.

Keywords *Arundo donax* L. · AMF inoculation · Substrate-induced respiration · Wastewater sludge compost · Soil enzymatic activity

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Introduction

Because of the climate crisis and regularized conventional agricultural practices, cultivated soils are increasingly deteriorating, losing their fertility, water holding and CO2 mitigating capacity (Borrelli et al. 2020). A promising approach would be to apply a cost-effective, environmentally friendly long-term solution to mitigate these negative tendencies using by-products of developing industries. Wastewater treatment is a quickly developing industry all over the world. Larger and larger quantities of wastewater are getting treated, producing increasing amounts of residuals, like sewage sludge (Kominko et al. 2017; Rév et al. 2017). Sewage sludge of the communal wastewater treatment is a practical raw material for biomass energy production (digestion for biogas production, pyrolysis) and for composting, which eliminates the pathogenic organisms (Cieslik et al. 2015; Kumar et al. 2017). However, disinfected (composted, heat-dried, digested or stabilized by clay minerals) wastewater sludge that still contains potentially toxic heavy metal ions has hardly been used for agricultural applications in a large scale (Yang et al. 2015). Furthermore, it is regulated by the EU in the frame of Council Directive 86/278/EEC (Gubišová et al. 2020). However, in lower amounts, it may be suitable for soil amendment due to the high concentration of nitrogen, phosphorus and microelements, its high potential for soil texture remediation, and the beneficial effects on both the composition and activity of the soil microbiome (Baldantoni et al. 2020; Uzinger et al. 2020; Samara et al. 2023). Therefore, disinfected sewage sludge could be used in an effective way for the improvement of low-fertility soils (Almási et al. 2025).

High-fertility soils, such as chernozem, are mainly used to grow food crops. From the perspective of human health, it would be hazardous and irresponsible to apply sewage sludge in these soils, as they may still contain potentially toxic heavy metal ions (Gubišová et al. 2020). Some crops for biomass energy production, such as giant reed (*Arundo donax* L.), silvergrass (*Miscanthus sinensis*) and switchgrass (*Panicum virgatum*), can grow on low-fertility soils (Lewandowski et al. 2003). It is now widely recognized that renewable energy sources are becoming crucial in mitigating dramatic climate change, especially during time of global energy crisis. Therefore, it would be highly desirable to develop suitable methods for producing substrates for biomass-based energy and to use the low-fertility soils for this purpose.

Arbuscular mycorrhizal fungi (AMF) play a key role in improving crop nutrition in low-fertility soils (Takács et al. 2018). They promote the uptake of essential elements such as nitrogen (N), phosphorus (P) and potassium (K), produce glomalin and improve the formation of water-stable soil aggregates (Fall et al. 2022; Jeffries et al. 2003). AMF can not only enhance plant growth but can also increase the ability of plants to tolerate biotic and abiotic stress conditions (Begum et al. 2019; Diagne et al. 2020; Mayer et al. 2017). However, the effect of AMF on the host's uptake of heavy metals (HMs) is contradictory. As it was previously described, both metal immobilization and translocation depend on the soil metal concentration, the types of the soil, the metal and the host species (Dhalaria et al. 2020; Negrini et al. 2022; Riaz et al. 2021). So far, no experiments have been conducted to determine the translocation mechanisms of AMF, although it is presumed that this occurs in fungal vacuoles (Ferrol et al. 2016). The following immobilization mechanisms have been revealed: incorporation of the vacuoles of the extracellular hyphae, activation of genes of the plants responsible for adsorbing heavy metal ions on the surface of the roots, adsorption of hyphae, chelate forming and complex forming by glomalin (Dhalaria et al. 2020; Ferrol et al. 2016; Riaz et al. 2021).

Giant reed is considered a suitable candidate for various experiments related to wastewater sludge compost (WSC) due to the following properties: (1) It is able to grow on low-fertility soils (Oginni and Singh 2019), (2) it is potentially a high source of lignocellulosic biomass (Gubišová et al. 2016), which is the substrate of second-generation bioethanol production, and (3) it has a high potential for accumulating certain potentially toxic HMs, like Cu and Zn (Gubišová et al. 2020; Simon et al. 2013). Although giant reed seems to have little dependence on AMF (Ondreičková et al. 2019), it has been established that AMF can enhance its tolerance to salt and HM (Dhalaria et al. 2020; Romero-Munar et al. 2019).

According to Luna et al. (2011), WSC has a positive effect on the number of natural AMF spores in the rhizosphere of maize. Another research project aimed at investigating the effect of wastewater sludge on the composition of the AMF community on the roots of giant reed (Ondreičková et al. 2019). The combined effect of some organic amendments and AMF in protecting pea plants from the toxic effects of heavy metal ions originating from wastewater-polluted soil has also been demonstrated (Farhad et al. 2024). However, there is currently no information available regarding the combined effect of WSC and AMF on the growth and physiological characteristics of giant reed plants.

We hypothesized that (1) the application of WSC may supply giant reed seedlings with macro- and micronutrients in early plant development, (2) the organic matter added with composts may improve water holding capacity of the soil and the microbial activity of the rhizosphere in a nutrient-poor acidic sandy soil, and (3) the benefits of wastewater sludge compost can be enhanced by inoculating selected AMF strains, which may mitigate the negative effects of WSC containing potentially toxic heavy metal ions. Based on these hypotheses we examined the effect of low-dose, commercially available WSC (15 t ha^{-1}) treatment with or without AMF inoculation on the nutritional status, heavy metal concentrations and the rhizosphere activity of giant reed (*Arundo donax* L.) plants in a short-term pot experiment.

Materials and methods

Altogether 40 giant reed (*Arundo donax* L. var. BL clone SC Blossom) plants were cultivated for a pot experiment. The plantlets were reproduced by sterile micropropagation according to the patent of US 7,303,916 B2 (Czakó and Márton 2007), in the laboratory of Arundo Cellulóz Farming Ltd (Hungary). Arundo plantlets were later acclimatized within greenhouse conditions in plantlet trays for 15 weeks.

The nutrient-poor acidic sandy soil (experimental site of the Institute for Soil Sciences, Centre for Agricultural Research in Nyírlugos, Hungary (47°43'N, 22°00'E)) used in the pot experiment and soil samples were taken from the plowed layer (0–30 cm). The particle size distribution of the soil was > 0.05 mm: 85%, 0.05–0.002 mm: 10%, < 0.002 mm: 5%.

The wastewater sludge compost (WSC) was a commercial product of Nyírségvíz Ltd. (Nyíregyháza, Hungary), composted at mesophilic conditions (55 °C). Chemical characterization of experimental soils and sewage compost is presented in Table 1. Ecotoxicological test of the wastewater sludge compost was taken by the Hungarian Standard (MSZ 21976-17:1993) method, which is based on the sprouting ability of seeds of white mustard (*Sinapis alba* L.) (Fig. 1). AMF strains originated from the European Bank of Glomales (BEG): (AMF1: *Funneliformis mosseae* (Schüssler and Walker); syn. *Glomus mosseae* (Nicholson and Gerdeman); BEG12), AMF2: *Funneliformis geosporum* (Nicolson and Gerdeman); BEG11), AMFmix: 50–50% mixture of the two AMF strains. All strains were propagated on cucumber grown in pumice. Cucumbers were cultivated in a growth chamber under controlled light and temperature conditions. As inoculum, a mixture of pumice and AMF-infected roots (with a colonization frequency F = 100% and mycorrhizal intensity M% = 89.5%) was used. The AMF inocula were applied at a rate of 20 g per pot, as a thin layer at a depth of 5 cm below the plant in the pots.

Table 1 Physical and chemical properties of the acidic sandy soil (Nyírlugos, Hungary) and wastewater sludge compost (Nyírségvíz Ltd.; Hungary)

Measured physical and chemical parameters	Soil	Compost
pH (H_2O)	4.8	5.1
OM (organic matter) %	0.5	36.7
$CaCO_3$ (mg kg^{-1})	0.0	38,200
Total N%	0.03	5.7
Total/AL-extractable plant-available element concentrations (mg kg^{-1})		
P	216/115	26,347/23,137
K	839/190	8317/8233
Total/LE-extractable plant-available element concentrations (mg kg^{-1})		
As	*	15.4/2.8
Cd	*	0.6/0.6
Co	2./0.2	3.3/0.1
Cr	2.2/*	39.7/ 0.4
Cu	24/1.3	289.3/ 97.3
Hg	*	0.336/*
Mn	137.6/15.4	430.7/2270
Mo	0.3/0.041	11.7/0.2
Ni	3.01/0.1	49.5/10.6
Pb	2.3/1.3	19.2/7.3
Se	*	1.8/ *
Zn	13.5/0.5	1209.3/757.4

Total P, K, As and metals: aqua regia soluble; AL: ammonium lactate soluble; LE: Lakanen-Erviö soluble; *: below detection limit (total/LE); As: < 0.4/0.08 mg kg^{-1}; Cd: < 0.02/0.004 mg kg^{-1}; Cr: < 0.1/0.01 mg kg^{-1}; Hg: < 0.12/0.03 mg kg^{-1}; Se: < 0.6/0.12 mg kg^{-1}. The percent and mg kg^{-1} data do refer to dry weight material

Experimental setup and growth conditions

Plants were cultivated in a growth chamber for 9 weeks (63 days after plantation; DAP) at 26/17 °C day/night temperature and 16/8 h photoperiod at a photon flux density of 600 L µmol m^{-2} s^{-1} and with a relative humidity of 50–60%. Arundo plants were planted into 40 pieces of 1.25 dm^3 plastic pots (1 seedling/pot) containing 1.5 kg of sandy soil. The doses of the wastewater sludge compost (WSC) and the AMF inoculation in the WSC and/or mycorrhizal treatments were the same. The treatments were the following: (1) no addition (Control (C)); (2) non-inoculated wastewater sludge compost (WSC; 18.8 g pot^{-1} (0.5 m/m%; 15 t ha^{-1})); (3) wastewater sludge compost and inoculum of *F. mosseae* strain (WSC + AMF1*;* 20 g AMF inocula/pot); (4) wastewater sludge compost and inoculum of *F. geosporum* strain (WSC + AMF2); and (5) wastewater sludge compost and inoculum of AMFmix strain mixture (WSC + AMFmix) (n = 8). WSC and AMF treatments were applied from the first day of the 9-week growing period. 100 ml tap water pot^{-1} and from the 5th week of the cultivation 200 ml water pot^{-1} were added three times a week. For optimal nutrition, 60 ml pot^{-1} P-poor Hoagland nutrient solution was added weekly from the 5th week. The dry content of the wastewater sludge compost was determined after drying until mass constancy in an oven (105 °C; Memmert UN75, Memmert GmbH + Co. KG, Aeussere Germany). The dry content was 38.85%, and the 0.5 m/m% was calculated with the dry mass.

Pre-harvest investigations

Chlorophyll content (CCI; CCM-200 plus Chlorophyll Meter; ADC Bioscientific Ltd., USA) and maximum quantum efficiency of photosystem II (Fv/Fm) (OS30 p$^+$ fluorimeter; Opti-Sciences, Hudson, USA) were measured on the youngest fully expanded leaf.

The electrical capacitance of the root–substrate system (CR) was measured as parallel resultant capacity by a GW-8101G LCR equipment (GW Instek Co. Ltd, Taiwan), using 1 kHz frequency and 1 V voltage. A 6-mm-diameter, 15-cm-long acid-free steel bar was used as the soil electrode, and the plant electrode was clamped at 15 mm above the substrate level on the shoot. The clamp was fixed through a

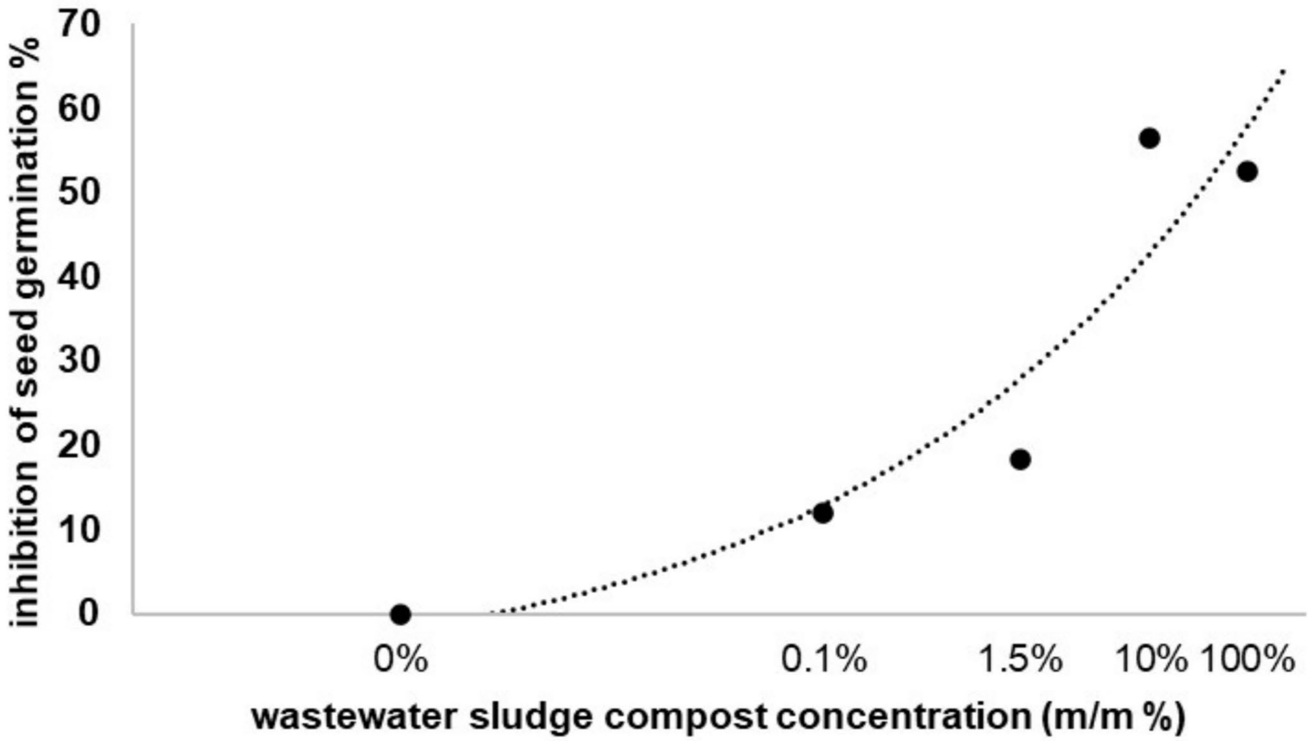


Fig. 1 Ecotoxicological test of the wastewater sludge compost (WSC)

5-mm-wide aluminum foil, and underneath that, we applied conductive gel (Vascotasin®; Spark Promotions Co. Ltd., Budapest, Hungary) to maintain good electric contact (Rajkai et al. 2005). The soil was watered to field capacity (0.232 cm^3 cm^{-3} (pF2.5)), and the moisture content of the upper layer (0–11 cm) was measured with a Trime-HD2 TDR meter (IMKO GmbH, Ettlingen, Germany), using a Pico electrode. The CR measurement was taken on the 55th day (DAP55) of the cultivation.

Post-harvest (destructive) measurements

Characterization of enzyme activities of rhizosphere soils, AMF colonization of the roots, biomass production (shoot and root dry weight; SDW and RDW) and leaf area of 10 per pot selected (the most well-conditioned) leaves (LA; LI-3100 Area Meter; LI-COR inc. Lincoln, Nebraska, USA) were accomplished.

MicroResp™ technique (Campbell et al. 2003) was used to characterize the community-level physiological profiles (CLPP) of the soils amended with wastewater sludge compost and AMF inoculation. Twenty-three substrates and ultrapure distilled water (control) in four replications (subsamples per plate) were distributed to each plate. The following substrates were used: L-arabinose (ARA), D-galactose (GAL), D-glucose (GLC), D-fructose (FRU) and trehalose (TRE), in 80 g L^{-1}; L-alanine (ALA), citric acid (CIT), L-lysine (LYS), DL-malic acid (MAL) and Na–succinate (SUC) in 40 g L^{-1}; L-arginine (ARG), 3.4-dihydroxybenzoic acid (DHB), L-glutamine (GLN) in 20 g L^{-1}. and L-glutamic acid (GLU) in 12 g L^{-1}; myoinositol (INO), D-mannitol (MAT), D-mannose (MAN), L-rhamnose (RHA), D-sorbitol (SOR) and D-xylose (XYL), in 80 g L^{-1}; L-asparagine monohydrate (ASN) in 20 g L^{-1} and D-gluconic acid potassium (GLA) and serine (SER) in 40 g L^{-1}. The pH of these substrate solutions was adjusted to 6.5 with 1 M NaOH or HCl solutions. Colorimetry was performed by reading the absorbance of the detection plates at the beginning and at the end of the 5 h 25 °C incubation at 570 nm (Biochrom Anthos 2010 Microplate Reader, Cambridge, UK). Respiration rates were then calculated from normalized % CO2 data after 5 h, using calibration raw, the ABS (absorbance) value was converted to v/v% and calculations were made for respiration rate [CO2 rate (Lig CO2-C g^{-1} h^{-1})]. To generate an index for total enzymatic activity ($\sum$SIR (total substrate-induced respiration)) of the soil microbiome, the single SIR values were summed across the plate.

In the root-free rhizosphere soils, dehydrogenase (Shaw and Burns 2006) and phosphatase (Öhlinger 1996) activities, fluorescein diacetate (FDA) hydrolysis assay (Adam and Duncan 2001), based on the method of Schnürer and Rosswall (1982), were measured by spectrophotometer (Hitachi

U2900; Hitachi High-Technologies Corporation, Tokyo, Japan; THERMOspectronic Helios P spectrophotometer, Artisan Technology Group Champaign, IL, USA). The soil samples were stored at 4 °C temperature until enzymatic measurements.

After harvesting, the root and the shoot samples were dried in an oven at 110 °C for 72 h until they reached a constant mass (Memmert UN75; Memmert GmbH + Co. KG, Aeussere Germany). The dry matter content of the WSC and soils was determined (105 °C, 4 h) before the enzymatic measurements and chemical analysis. The methods for the analysis of acidic sandy soil and WSC analysis were the same. The pH was measured in 1:2.5 soil–water suspension 12 h after mixing according to the Hungarian Standard (MSZ-08–0206/2:1978). The modified Walkley–Black method (MSZ-08–0452:1980) was used to determine the organic matter (OM %) content. The concentrations of plant-available P and K were determined after extraction with ammonium acetate lactate (AL-P2O5 and AL-K2O) (Egnér et al. 1960). The total N concentration was measured by the Kjeldahl method (ISO 11261:1995). The NH4-N and NO3-N concentrations were measured from KCl extracts according to the Hungarian Standard (MSZ 20135:1999).

The pseudo-total element concentrations (in soil, WSC and plant samples) were determined from the air-dried samples after acidic digestion in a Teflon bomb microwave (cc. HNO3 + cc. H2O2) extract (MSZ-21470–50:2006). The plant-available element concentrations were measured in ammonium acetate + EDTA extraction (LE) based on the method of Lakanen and Erviö (1971). Nitrogen concentration in the leaves was determined by the Kjeldahl method (1883) after the samples were digested in sulfuric acid (cc. H2SO4).

The concentrations of nutrients and potentially toxic elements were determined by inductively coupled plasma–atomic emission spectroscopy (ICP–AES; HORIBA Jobin Yvon Ultima-2 sequential instrument, France).

Roots were randomly sampled for the measurement of AMF colonization, which was performed after staining the separated root (< 1 mm in diameter) subsamples with lactic acid–aniline blue (according to Phillips and Hayman 1970). AMF colonization was estimated using a BX51 microscope (40–200 × magnification; Olympus Corp., Tokyo, Japan). The intensity of colonization (M %) and the arbuscular richness (A%) in the roots were calculated using a five-class system (Trouvelot et al. 1986).

Statistical analysis

One-way variance analysis (ANOVA) combined with the Tukey–Kramer multiple comparison post hoc test was performed using InStat software (GraphPad Software Inc.) to determine the significance of the differences between the results from different treatments. The quantitative report of the statistical analysis is summarized in Table 2 Supplementary. Principal component analysis (PCA) was taken to detect the patterns in respiratory activity among the substrates. The PCA was performed using R-statistic software (R Core Team 2019).

Results

According to the ecotoxicological test, the wastewater sludge compost had a toxic effect (Fig. 1).

The x-axis shows the wastewater sludge compost concentration of the liquid used for treatment of the seeds. The inhibition % is the percentage of the non-germinating seeds after 72 h. The m/m% was calculated with the fresh weight of the WSC.

A strong inhibitory effect of the sludge compost was observed on the germination of *Sinapis alba* L. at the two highest concentrations of WSC. In the case of the application of 10% wet compost, the phytotoxicity of the water-soluble components caused a toxicity higher than the EC_{50} value during germination.

Following the treatment with composted wastewater sludge, the root electrical capacitance (CR) became higher; in addition, it was higher also for the AMF-inoculated plants (Fig. 2). However, no significant differences in CR were found between the AMF-inoculated plants.

Mean values and standard deviation were represented (n = 8). Different letters indicate means that were significantly different at $P < 0.05$. The significance of the differences between the results from different treatments was determined based on one-way variance analysis (ANOVA) combined with the Tukey–Kramer multiple comparison post hoc test. C: control; WSC: wastewater sludge compost treated; WSC + AMF1: wastewater sludge compost treated and inoculated by AMF1 strain (*Funneliformis mosseae*); WSC + AMF2: wastewater sludge compost and inoculated by AMF2 strain (*F. geosporum*); WSC + AMFmix: wastewater sludge compost treated and inoculated by the mixture of AMF1 and AMF2 (1:1).

No significant differences were observed in the results of the in situ phenotype analysis (including height, number of branches and nodes, and number of leaves) between treatments, and no differences in maturation tendencies were evident (data not presented).

In repeated measurements, no significant differences in CCI and Fv/Fm values were found between different WSC and AMF treatments (Fig. 3). Before harvest (DAP61) CCI ranged from 16.44 ± 3.35 to 22.76 ± 1.51, the control had the lowest rate. The lowest Fv/Fm values (0.785 ± 0.0) were measured in the WSC-treated plants.

Table 2 Concentrations of the nutrients and potentially toxic elements (mg kg^{-1} dry weight) in the roots of the *Arundo donax* L. treated by wastewater sludge compost and AMF

Treatments elements	C	WSC	WSC + AMF1	WSC + AMF2	WSC + AMFmix
N (m/m%)	0.76 ± 0.07 a	0.82 ± 0.02 a	0.78 ± 0.04 a	0.82 ± 0.04 a	0.89 ± 0.06 b
P (mg kg^{-1})	1027.71 ± 333.71 a	1616.87 ± 492.64 b	1194.39 ± 66.86 ab	1383.63 ± 269.76 ab	1508.50 ± 328.37 b
K (mg kg^{-1})	10,607.98 ± 2270.34 a	13,237.53 ± 1156.21 b	14,744.23 ± 1331.79 b	15,081.05 ± 839.68 b	12,777.83 ± 1533.43 b
As (mg kg^{-1})	0.46 ± 0.27 a	0.55 ± 0.27 a	0.38 ± 0.05 a	0.44 ± 0.16 a	0.40 ± 0.09 a
Cd (mg kg^{-1})	0.27 ± 0.10 a	0.20 ± 0.04 a	0.16 ± 0.03 a	0.17 ± 0.08 a	0.18 ± 0.06 a
Co (mg kg^{-1})	3.33 ± 1.03 a	2.78 ± 0.89 a	1.56 ± 0.37 a	1.67 ± 0.68 a	2.51 ± 1.13 a
Cr (mg kg^{-1})	3.94 ± 1.07 a	5.25 ± 1.16 a	4.72 ± 1.07 a	5.16 ± 1.74 a	4.84 ± 1.43 a
Cu (mg kg^{-1})	23.98 ± 6.93 ab	30.37 ± 8.05 b	20.50 ± 2.73 a	20.61 ± 5.52 a	27.93 ± 6.64 b
Mn (mg kg^{-1})	740.71 ± 235.42 b	347.69 ± 215.51 a	161.04 ± 45.87 a	192.05 ± 83.14 a	377.83 ± 270.74 a
Mo (mg kg^{-1})	0.92 ± 0.13 a	1.02 ± 0.22 a	0.80 ± 0.15 a	0.89 ± 0.25 a	1.00 ± 0.23 a
Ni (mg kg^{-1})	2.84 ± 0.50 a	4.28 1.05 a	3.10 ± 0.39 a	3.12 ± 0.91a	3.30 ± 1.07a
Pb (mg kg^{-1})	2.05 ± 0.71 c	1.71 ± 0.58 cb	0.30 ± 0 a*	0.57 ± 0.50 a	0.94 ± 0.86 ca
Zn (mg kg^{-1})	56.37 ± 18,30 a	94.55 ± 21.84 b	62.36 ± 12.59 a	66.12 ± 21.14 a	79.86 ± 21.88 ab

Mean values (numbers before ±) and standard deviations (numbers after ±) were represented (n = 8). Different letters indicate means that were significant differences at $P < 0.05$. To determine the significance of the differences between the results from different treatments was based on one-way variance analysis (ANOVA) combined with the Tukey–Kramer multiple comparison post hoc test. C: control; WSC: wastewater sludge compost treated; bold letters indicate the significant differences between treatments. WSC + AMF1: wastewater sludge compost treated and inoculated by AMF1 strain (*Funneliformis mosseae*); WSC + AMF2: wastewater sludge compost and inoculated by AMF2 strain (*F. geosporum*); WSC + AMFmix: wastewater sludge compost treated and inoculated by the mixture of AMF1 and AMF2 (1:1); *: The variance was low; we described it until two decimal places. It was 0.002

The leaf area was significantly larger in the case of the plants inoculated by AMF1. There were no significant differences in the biomass measurements (Fig. 4) or the root/shoot dry mass ratio (data not shown).

The pattern of substrate utilization evidently differed in the case of samples inoculated with AMF2 especially to the direction of glutamine (GLN) and glutamate (GLU) (Fig. 5; based on the results marked in the Table 1 supplementary). In the case of 14 substrates of the total 23 of substrate-induced respiration (SIR) tests, the AMF2-treated samples showed prominently the strongest values (Table 1 Supplementary) though only 5 of them are significantly different. Only the AMF2-inoculated samples showed significantly distinctive values in the 3.4-L-glutamine (GLN), L-glutamic acid (GLU), DL-malic acid (MAL), Na–succinate (SUC) and dihydroxybenzoic acid (DHB) (Fig. 6a-e).

No significant differences were found in the values of the total substrate utilization (total SIR; ∑SIR) (Fig. 6f).

In two tests related to enzymatic activity, the rhizosphere of AMF-inoculated plants exhibited higher activity levels than uninoculated plants (Fig. 7). No difference was detected between the control and WSC treatments for dehydrogenase, while the AMF-inoculated samples showed significantly higher values than the WSC treatment (Fig. 7a). The difference was lower in the case of AMFmix-inoculated samples than in monoculture-inoculated samples (AMF1 and AMF2). In the case of phosphatase, the significance was observed only at AMF2-inoculated samples comparing to both control and uninoculated, WSC-treated samples

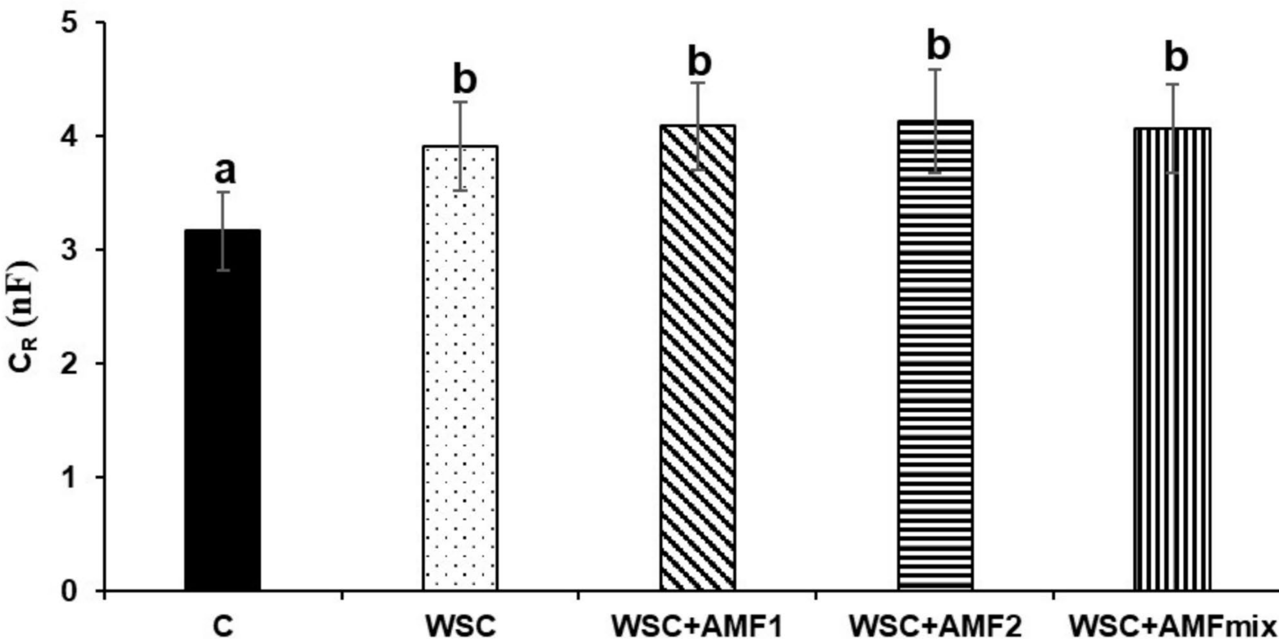


Fig. 2 Electrical capacitance of the root–substrate system (CR) of *Arundo donax* L. treated with wastewater sludge compost (WSC) and different AMF strains on the 55th day after plantation

(Fig. 7b). No significant difference was observed among the samples in the fluorescein diacetate assay (FDA) (Fig. 7c).

The results of the root analysis (Table 2) showed more significant differences in the element concentration between the treatments than the results of shoot analysis. We did not find relevant differences among treatments in the N concentration of the roots, except the WSC + AMFmix-treated plants showed a significantly higher value. The root P concentration increased as a result of WSC treatment, and that difference decreased in the case of monocultural AMF-inoculated plants; in the case of WSC + AMFmix-treated soil samples, a similar difference was observed. The K concentration of the roots was significantly higher at all the WSC-treated plants, and no difference was measured between the AMF-uninoculated and AMF-inoculated ones. Most of the heavy metal (HM) concentrations showed lower values in the AMF-inoculated plants, although the differences were not always significant. Mn concentrations of the roots were significantly lower in all WSC treatments, at both the not inoculated and the inoculated ones. In the case of Zn, Cu and Pb, the AMF-inoculated plants showed significantly lower metal concentrations compared to the non-inoculated, WSC-treated ones. The AMFmix-inoculated plants showed fewer differences than the monocultural inoculated ones, compared to the not inoculated plants (Table 2).

No significant differences were observed among the treatments with regard to N and K concentrations in the shoots (Table 3). The P concentration of the shoot was significantly higher in all WSC-treated plants, with no difference was measured between the non-inoculated and inoculated ones. Of the HMs only Mn and Zn concentrations differed significantly in the shoots. As with root analysis results, Mn concentrations were lower in the WSC-treated plants. Additionally, the AMF-inoculated, WSC-treated samples (only the monocultural ones), resulted in significantly lower values than the non-inoculated, WSC-treated plants. The Zn concentration was significantly higher in both the inoculated and the uninoculated, WSC-treated samples than in the control plants. There were no differences in Cu concentration between the treatments (Table 3).

No significant differences in AMF colonization parameters (M % and A%) were observed among the root samples from different treatments. This indicates that the control and the non-inoculated, WSC-treated plants were colonized by indigenous AMF from the experimental soil. The intensity of mycorrhizal colonization (M%) in the roots ranged from 39.2 ± 9.6% (WSC + AMF2) to 48.1 ± 13.19% (WSC + AMFmix) among the different treatments. The M% of Arundo roots colonized by indigenous AMF exhibited a 7% increase in response to WSC. The arbusculum richness ranged from 9.8% ± 6.4% (WSC + AMF2) to 20.01% ± 10.7% (WSC).

Discussion

Among energy crops, the rapidly growing, high biomass productive and resistant giant reed (*Arundo donax* L.) can be successfully cultivated on low-fertility soils treated with

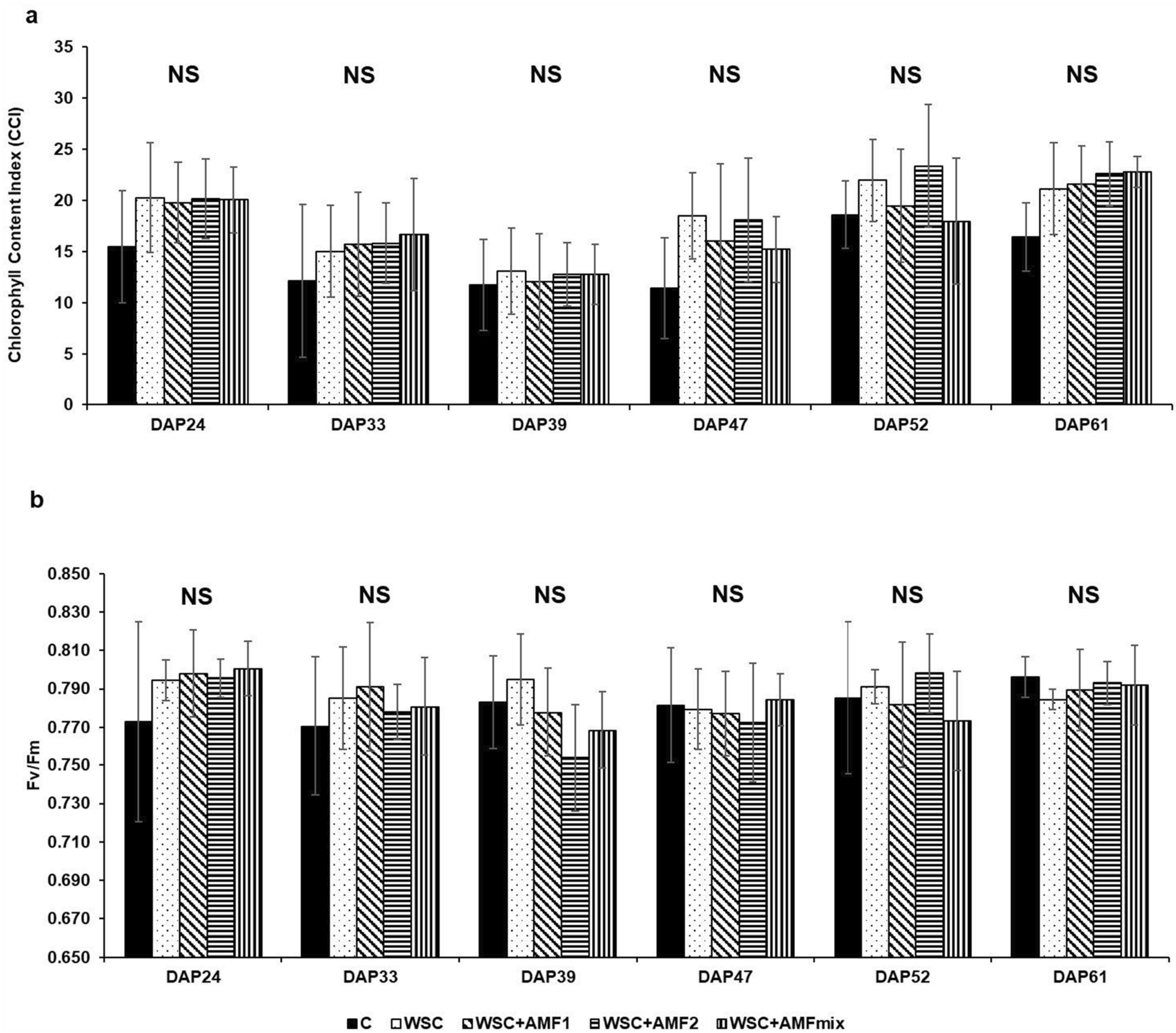


Fig. 3 **a**-**b** Leaves chlorophyll content (CCI) and PSII photochemical efficiency (Fv/Fm) of *Arundo donax* L. treated with wastewater sludge compost (WSC) and different AMF strains **a**: Chlorophyll Content Index (CCI) **b**: maximal quantum efficiency of PS II (Fv/Fm). Mean values and standard deviation were represented (n = 8). DAP: Day after planting. NS: No significance was observed among the treatments within one DAP. C: control; WSC: wastewater sludge compost treated; WSC + AMF1: wastewater sludge compost treated and inoculated by AMF1 strain (*Funneliformis mosseae*); WSC + AMF2 (*F. geosporum*): wastewater sludge compost and inoculated by AMF2 strain; WSC + AMFmix: wastewater sludge compost treated and inoculated by the mixture of AMF1 and AMF2 (1:1); NS: No significant difference

N-, P- and K-rich sewage sludge compost (Simon et al. 2013). According to the results of WSC-related experiments with giant reed, the dose of the WSC we applied was still nutritional, but not yet stressful for giant reed, as previous research demonstrated (Gubišová et al. 2020). Gubišová et al. (2020) conducted a short-term pot experiment on Luvi-Haplic Chernozem soil that had been treated with two types of anaerobically digested sewage sludge applying doses of either 5 or 15 t ha^{-1}. The biomass and concentrations of Cu and Zn in the shoots of Arundo increased, depending on the type and dose of sewage sludge. The total elemental composition of the WSC applied in this study was resembled that of communal wastewater sludge (Singh et al. 2010), except for the somewhat higher amount of phosphorus (Table 1). The high amount of WSC addition can cause N–P imbalance in the soil, making the availability of the resources less favorable for the AMF–plant interactions (Reimer et al. 2023). Arbuscular mycorrhizal fungi are sensitive to the high levels of phosphorus in soil or the growth media of the host (Thomson et al. 1986). Given this, the combined

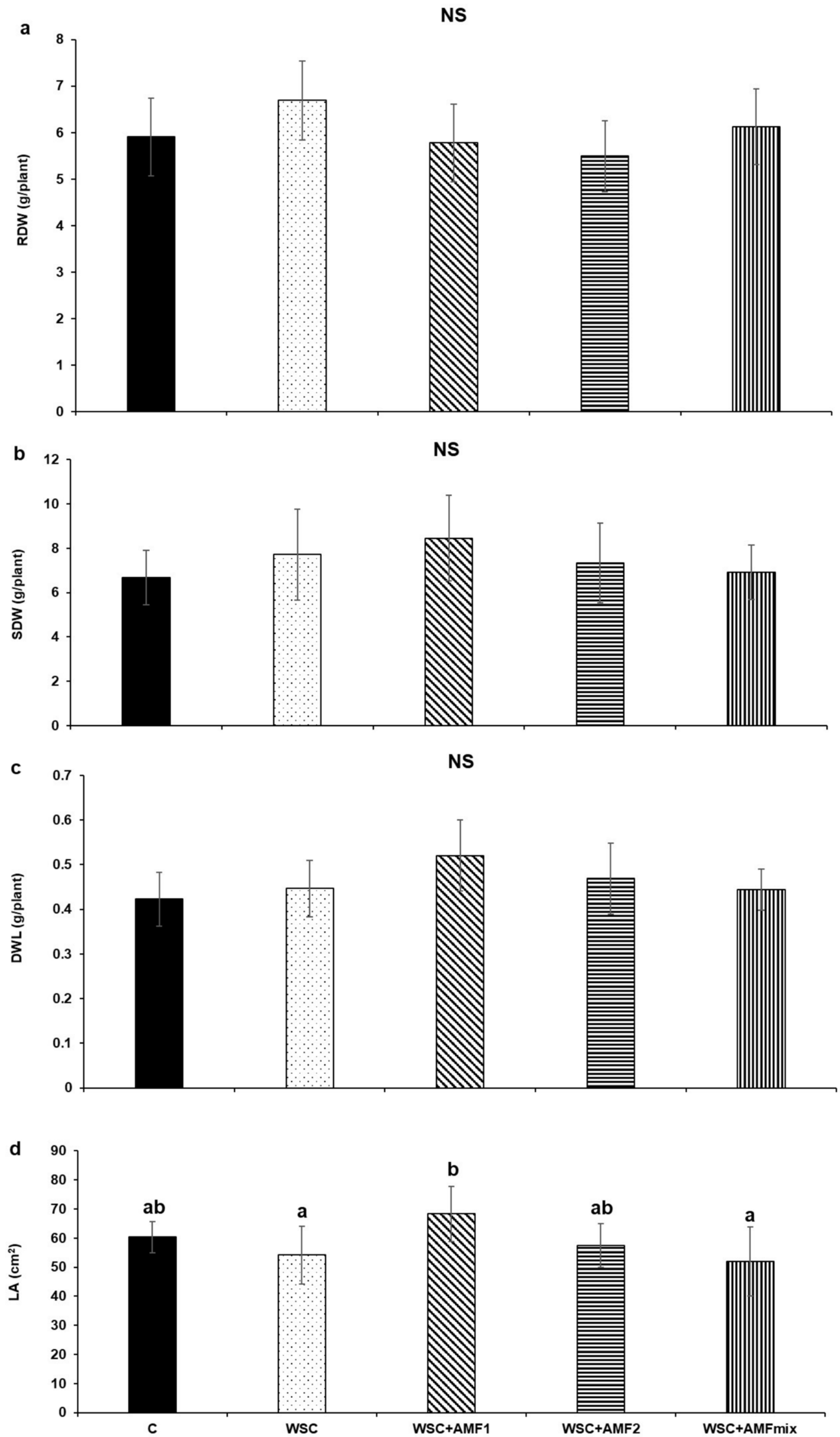


Fig. 4 **a**-**d** Dry weights and leaf area of *Arundo donax* L. treated with wastewater sludge compost (WSC) and different AMF strains **a**: Dry weight of the Arundo roots (RDW); **b**: dry weight of the shoots (SDW); **c**: dry weight of the selected 10 leaves (DWL); **d**: leaf area of the selected 10 leaves (LA). Mean values and standard deviation were represented (n = 8). Different letters indicate means that were significant differences at $P < 0.05$. To determine the significance of the differences between the results from different treatments was based on one-way variance analysis (ANOVA) combined with the Tukey–Kramer multiple comparison post hoc test. C: control; WSC: wastewater sludge compost treated; WSC + AMF1: wastewater sludge compost treated and inoculated by AMF1 strain (*Funneliformis mosseae*); WSC + AMF2: wastewater sludge compost and inoculated by AMF2 strain (*F. geosporum*); WSC + AMFmix: wastewater sludge compost treated and inoculated by the mixture of AMF1 and AMF2 (1:1); NS: No significant difference

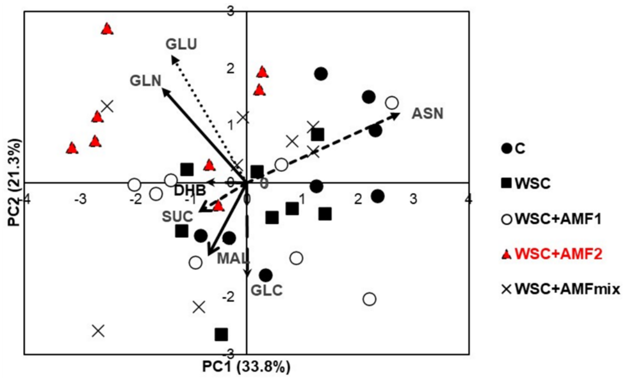


Fig. 5 Principal component analysis (PCA) for the MicroResp.™-based microbial community-level physiological profiling (CLPP) in the wastewater sludge compost and AM fungal-treated acidic sandy soil of Arundo donax L. (Table 1 supplementary). The arrows indicate the PCA percentage of the utilized substrates, which utilization rates showed differences between treatments, and/or one of the vector components is higher than 1.00. The presented substrates: D-glucose (GLC), DL-malic acid (MAL), Na–succinate (SUC), 3.4-dihydroxybenzoic acid (DHB), L-glutamine (GLN), L-glutamic acid (GLU), L-asparagine monohydrate (ASN) and serine (SER). C: control; WSC: wastewater sludge compost treated; WSC + AMF1: wastewater sludge compost treated and inoculated by AMF1 strain (*Funneliformis mosseae*); WSC + AMF2: wastewater sludge compost and inoculated by AMF2 strain (*F. geosporum*); WSC + AMFmix: wastewater sludge compost treated and inoculated by the mixture of AMF1 and AMF2 (1:1)

use of additives rich in P and the AMF inoculation must be approached very carefully. The low-dose treatment of WSC on low-fertility soil improved the nutrition (K, P concentration in the shoots) of the giant reed (Tables 2, 3) and also increased the adsorptive surface area of the roots. This was demonstrable by measuring of electric capacity of the root–substrate system (Fig. 2). Neither growth nor nutrition parameters proved the WSC–AMF synergism (Figs. 2, 4). It was presumed that the active surface of the roots was smaller due to the nutrient limitation in the case of control samples (Cseresnyés et al. 2014). Although no significant difference in root dry weight was measured, the values of WSC-treated plants were higher, while the values of WSC + AMF1 and WSC + AMF2 plants were lower than of the control (Fig. 6a). Therefore, the elevation of CR in WSC treatments demonstrated the effect of WSC supplementation alone on the soil nutrition, increasing the active surface area of the root system. In contrast, the increase in AMF-inoculated plants demonstrated the combined effect, where the active surface area of the roots was lower, but the extra-radical hyphae system may have overcompensated this difference. The fact that the difference of the dry weight of the roots was not significant, yet the difference of CR was significant, may demonstrate the sensitivity of the root capacitance method. Neither the aboveground, nor the belowground dry biomass measurement showed relevant effects of AMF or WSC (Fig. 4). Only the leaf area was significantly higher in the case of AMF1-inoculated plants compared to both the not inoculated and WSC-treated and the AMFmix-inoculated ones (Fig. 4). Similar experiments were performed on common reed, but the AMF treatment decreased both the aboveground and belowground parts of the plants, while increasing the leaf area, as observed in our experiment (Liang et al. 2018). This provided little evidence of the positive effects of AMF1 inoculation.

One of the aims of the research was to demonstrate the effect of plus addition of selected AMF inoculants on soil microbial activity in the mycorrhizosphere of *Arundo donax*. The MicroResp™ technique is a sensitive method for detecting the community-level physiological profile (CLPP) differences in the soil caused by AMF inoculation (Gao et al. 2022). Our study appears to be the first to demonstrate this method to detect the CLPP changes induced by a combined treatment of WSC and AMF. Since the *F. mosseae* and the *F. geosporum* strains are highly infectious, and the infectivity of the native AMF community of the acidic sandy soil of Nyírlugos is low according of a previous study (Rékási et al. 2019), and the homogenized soil we applied in the experiment was root-free, the results indicated that the inoculation of the two AMF strains had different effects on the host rhizosphere (Figs. 5, 6). In our experiment, the effects of the AMF on the giant reed are applied strain-specific and dependent on the composition and competition of the present AM fungal community. The AMF2-inoculated soils showed significantly different activities than the soils treated with AMF1 or AMFmix (Fig. 6). Three soil hydrolytic enzyme activity-related tests were conducted including INT-based dehydrogenase activity, while in the course of previous works mostly the 2,3,5 triphenyl tetrazolium chloride (TTC)-based dehydrogenase activity was measured (Jabborova et al. 2021; Qiu et al. 2019). In the case of all these tests, the AMF-inoculated soils showed higher enzymatic activity than the uninoculated ones (Fig. 7). Based on the soil enzymatic measurements taken during our research, we can safely conclude that inoculation, particularly with AMF2, increases microbial activity in the acidic sandy soil. Similarly, an earlier study has already demonstrated that the AMF inoculation increases the values of soil enzyme-related tests, such as FDA assay and TTC-based dehydrogenase activity, and the microbial biomass in the mycorrhizosphere (Jabborova et al. 2021). Although several previous reports have shown the positive effects of AMF inoculation on the parameters related to soil microbial activity (Qiu et al. 2019; Yang et al. 2011), according to available information no experiment involving giant reed has yet been conducted. The most frequently measured parameters among these experiments are soil enzyme activity (TTC), glomalin content and soil organic carbon (SOC). A review article summarizing the results of 341 measurements from

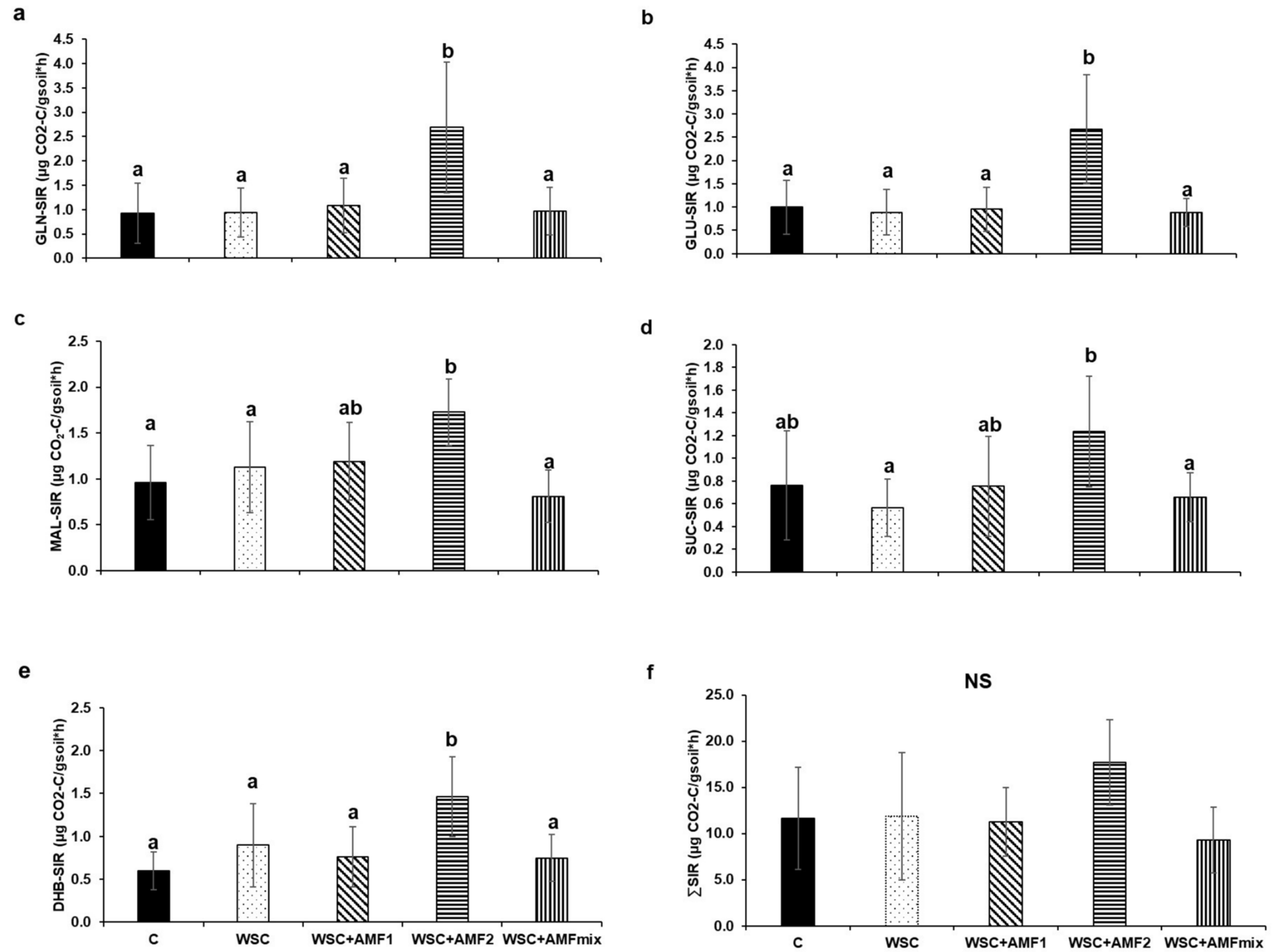
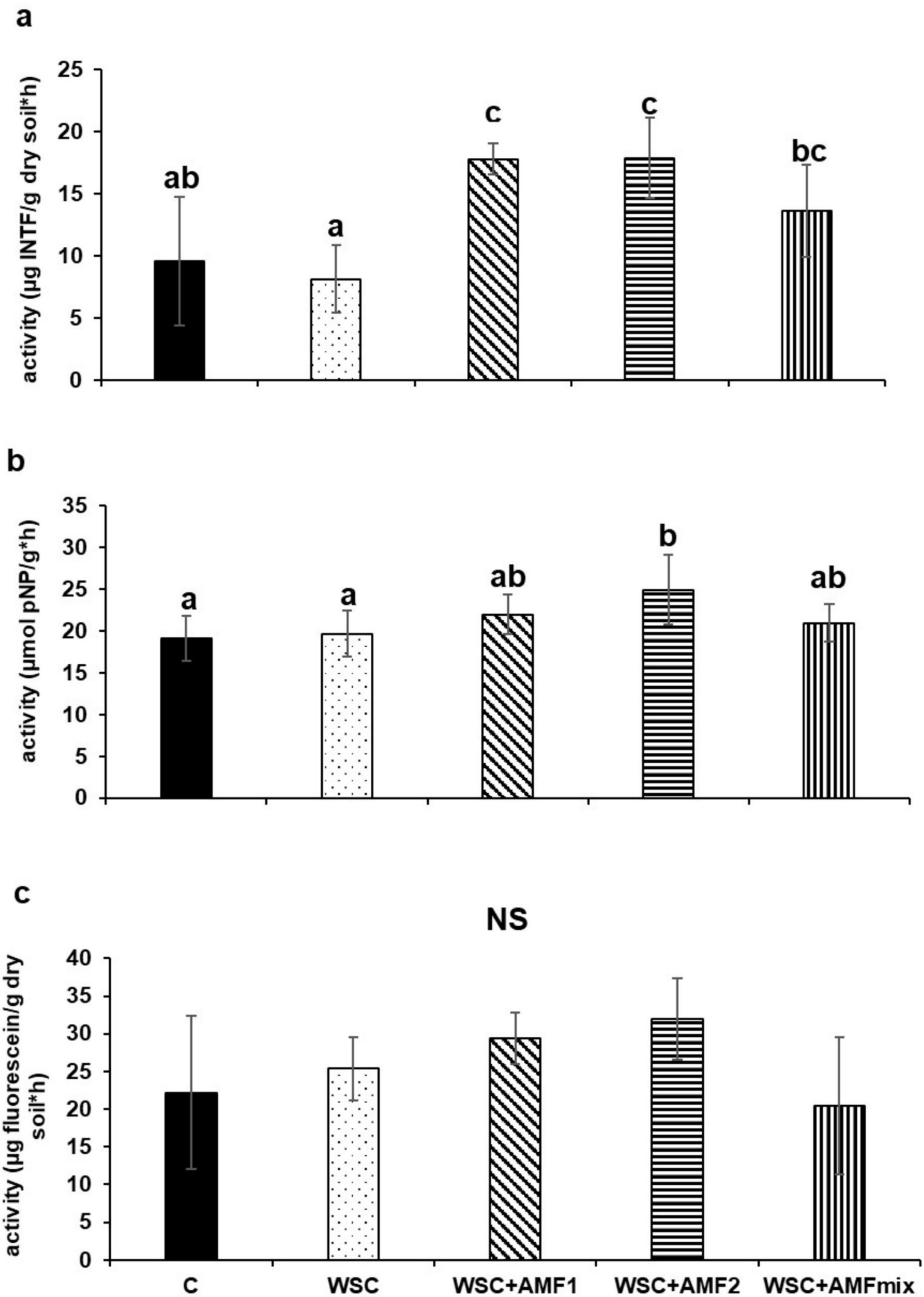


Fig. 6 **a**-**f** Substrate-induced respiration (SIR) of L-glutamine (**a**), L-glutamic acid (**b**), DL-malic acid (**c**), Na–succinate (**d**), 3,4-dihydroxybenzoic acid (**e**) and total utilization of all of the substrates (**f**). **a**: GLN: utilization of L-glutamine; **b**: GLU: utilization of L-glutamic acid; **c**: MAL: utilization of DL-malic acid; **d**: SUC: utilization of Na–succinate; **e**: DHB: utilization of 3.4-dihydroxybenzoic acid; f: ∑SIR: SIR: total utilization of all of the substrates. Mean values and standard deviation were represented (n = 8). Different letters indicate means that were significant differences at $P < 0.05$. To determine the significance of the differences between the results from different treatments was based on one-way variance analysis (ANOVA) combined with the Tukey–Kramer multiple comparison post hoc test. C: control; WSC: wastewater sludge compost treated; WSC + AMF1: wastewater sludge compost treated and inoculated by AMF1 strain (*Funneliformis mosseae*); WSC + AMF2: wastewater sludge compost and inoculated by AMF2 strain (*F. geosporum*); WSC + AMFmix: wastewater sludge compost treated and inoculated by the mixture of AMF1 and AMF2 (1:1); NS: No significant difference

56 research papers describes the effects of AMF inoculation on the soil's separate hydrolytic enzyme activity, including benzoyl-L-arginine-amide (BAA)-hydrolyzing protease. Urease, nitrogenase, phosphatase (acidic, alkaline, neutral and total), cellulase, chitinase, esterase, invertase, trechalase, P-glucosidase, catalase, dehydrogenase, peroxidase, fluorescein diacetate (FDA) hydrolyzing enzymes and phenol oxidase. AMF inoculation was found to have a positive effect on all enzyme activities, except polyphenol oxidase (Qin et al. 2020).

As no difference in N concentration was found in either the roots or the shoots, it could mean that the giant reed is able to regulate the minimal N concentration of its own tissues (Tables 2, 3). The K concentration of the roots was lower only in the case of control plants, and this difference was compensated for the shoots, which may be the result of a regulating mechanism in the plant. Soil acidity can promote metal bioavailability (Wang 2015; Adamczyk-Sabela 2015). Our experiment also enabled us to observe and demonstrate that the HM concentrations were lower in the roots of the inoculated plants. The plantlets inoculated by *F. mosseae* (AMF1-inoculated samples) exhibited the lowest values. Our research suggests that the AMF fungi protect the plants from the potentially toxic effect of the HMs by such as Cu, Zn and Pb by decreasing their concentrations without dilution effect. The Pb concentrations in the roots were significantly


a
activity (µg INTF/g dry soil*h)
25
20
15
10
5
0
ab
a
c
c
bc
b
activity (µmol pNP/g*h)
35
30
25
20
15
10
5
0
a
a
ab
b
ab
c
NS
activity (µg fluorescein/g dry soil*h)
40
35
30
25
20
15
10
5
0
C
WSC
WSC+AMF1
WSC+AMF2
WSC+AMFmix

◀**Fig. 7** **a**-**c** Enzymatic activity in dehydrogenase (**a**), phosphatase (**b**) and fluorescein diacetate hydrolysis tests (**c**) of the wastewater sludge compost and AM fungal-treated acidic sandy soil of *Arundo donax* L. **a**: activity of dehydrogenase; **b**: activity of phosphatase; **c**: fluorescein diacetate hydrolysis test. Mean values and standard deviation were represented (n = 8). Different letters indicate means that were significant differences at $P < 0.05$. To determine the significance of the differences between the results from different treatments was based on one-way variance analysis (ANOVA) combined with the Tukey–Kramer multiple comparison post hoc test. C: control; WSC: wastewater sludge compost treated; WSC + AMF1: wastewater sludge compost treated and inoculated by AMF1 strain (*Funneliformis mosseae*); WSC + AMF2: wastewater sludge compost and inoculated by AMF2 strain (*F. geosporum*), WSC + AMFmix: wastewater sludge compost treated and inoculated by the mixture of AMF1 and AMF2 (1:1). NS: No significant difference

lower, and it were even undetectable in the shoots at all treatments. Despite the previous results, no difference was observed in the Cu, P and Zn concentrations in the shoots between inoculated and not inoculated, WSC-treated samples. This could be attributed to the increasing effect of AMF on the translocation of HMs from roots to shoots, as demonstrated in previous reports (Negrini et al. 2022; Pirsarandib et al. 2022) and review papers (Bhantana et al. 2021; Solís-Ramos et al. 2021). In a pot experiment, the application of Cu-adapted AM fungi mitigated the effects of Cu-polluted soils, resulting in an increased survival rate of *Oenothera picensis* growing in (Meier et al. 2015). In unpolluted soils the AM fungal treatment increased the root–shoot translocating factor of copper. Therefore, we can conclude that AM increases the translocating factor of Cu (as an essential element) in case of low concentration of Cu, while the Cu ions are immobilized in case of high Cu concentration. The low Mn concentrations in the roots and the shoots demonstrate that both WSC and AMF can immobilize this ion. The only significant difference in Mn concentrations between the control and WSC-treated plants was observed in the roots. Conversely, the difference in Mn concentrations between the non-inoculated, WSC-treated plants and the inoculated monocultural plants was also significant in the shoots. A previously published study demonstrated that the survival of poplar clones in HM-polluted areas is positively correlated with AMF colonization (Takács et al. 2005). Some previous experiments (Dhalaria et al. 2020; Riaz et al. 2021; Zhan et al. 2019) have demonstrated the mitigating effect of AMF on the uptake of HMs.

Throughout our experiments, the positive effects of *F. mosseae* (AMF1) were demonstrated by measurements of the soil enzymology, leaf area, root and shoot analysis and electrical capacitance. The positive effects of the strain *F. geosporum* (AMF2) were demonstrated through the measurements of soil enzymology, root and shoot analysis, substrate-induced respiration and the electrical capacitance (Figs. 2, 5, 6; Tables 2, 3, Table S1). No significant differences in HM immobilizing ability were observed between AMF1- and AMF2-inoculated samples for most of the HMs. *F. geosporum* prefers low-fertility, saline and sodic soils and gypsum rocks. *F. geosporum* isolates can tolerate a wide range of pH levels (Bothe et al. 2002; Kawahara et al. 2016) and an enormous potential to assist plant resistance under extreme soil conditions. It may be a reason, why this pH generalist AMF strain could have had a beneficial effect on the microbial activity in acidic sandy soil as shown by all kind of enzyme-related measurements (Figs. 6, 7). *F. geosporum* is the most effective as in low-fertility soils as in soils with extreme pH. In the present experiment, the effects of the inoculation with the mixture of the strains (AMF-mix) were generally lower than that of the monocultural inoculants.

The fungal colonization assessments demonstrated that neither the WSC nor the AMF treatment caused any changes in colonization rates, but the significant differences, resulted in the measurements of soil enzymatic-related tests and the element analysis of roots/shoots, were somehow caused by AMF. Previous research (Ondreičková et al. 2019) aimed at demonstrating the effect of the added sewage sludge (SS) on the colonization of indigenous AMF to the roots of giant reed, which demonstrated that a short-term SS treatment did not result in a significant shift in the composition of natural fungal community. That experiment convincingly showed that the most representative AMF groups—*Glomus* sp. and *Funneliformis* sp. strains—were present in both the control and treated samples. There was no report about the effect of AMF inoculation on the natural colonization of the rhizosphere of giant reed, but an experiment (Janousková et al. 2013) aimed at detecting the effect of AMF inoculation on natural AMF background by other plants like Medicago. It proved that the inoculation did not cause differences at colonization rate, and there was supposed to be an optimal AMF density of the soil/plant. Inoculation of new species may lead to increased competition between the strains and lowers the positive effect of the natural AMF community because of interspecific competition.

Based on the results of colonization test of our experiment, we could deduce that there must be also an optimal AMF colonization rate of the root of giant reed regulated by the plant. The plant-regulated colonization mechanism was demonstrated in cucumber by earlier research (Pinior et al. 1999). There might be two reasons for the measured differences caused by AMF inoculation: The differences in the enzymatic measurements might have been caused by the AMF strain-specific effects and by the different microbial composition of the mycorrhizosphere, although the results of MicroResp™ measurement demonstrated differences only in case of inoculation by AMF2. The differences in the HM concentrations of the root might have resulted from functional interactions among giant reed, AMF strains

Table 3 Concentrations of the nutrients and potentially toxic elements (mg kg^{-1} dry weight) in the shoots of the *Arundo donax* L. treated by wastewater sludge compost and AMF

Treatments elements	C	WSC	WSC + AMF1	WSC + AMF2	WSC + AMFmix
N (m/m%)	0.93 ± 0.15 a	0.9873 ± 0.12 a	0.90 ± 0.13 a	0.99 ± 0.14 a	1.03 ± 0.08 a
P (mg kg^{-1})	2398.25 ± 359,07 a	3527.9 ± 521.77 b	3452.74 ± 461.88 b	3328.59 ± 442.13 b	3651.30 ± 258.47 b
K (mg kg^{-1})	16,763.74 ± 2456.97a	17,912.74 ± 2828.70 a	18,844,36 ± 2027.42 a	19,156,52 ± 2432.66 a	19,878,96 ± 1675.91a
Cr (mg kg^{-1})	30.44 ± 6.36 a	26,5700 ± 8,23 a	21.65 ± 6.08 a	22.74 ± 5.56 a	24.42 ± 7.64 a
Cu (mg kg^{-1})	6,46 ± 0.95 a	6,5571 ± 0.88 a	6.51 ± 0.83 a	6.35 ± 0.86 a	7.18 ± 0.62 a
Mn (mg kg^{-1})	430.08 ± 65.86 c	343.5 ± 45.63 b	270.29 ± 42.37 a	259,77 ± 16,46 a	312.47 ± 62.96 ba
Mo (mg kg^{-1})	3.46 ± 0.82 a	3.0321 ± 1.08 a	2.49 ± 0.83 a	2.80 ± 0.65 a	2.79 ± 0.89 a
Ni (mg kg^{-1})	15.12 ± 3.08 a	13.4613 ± 4.23 a	11.20 ± 3.15 a	11.50 ± 2.88 a	12.42 ± 3.81 a
Zn (mg kg^{-1})	13.88 ± 3.50 a	32.4152 ± 6.67 b	34.29 ± 8.68 b	33,74 ± 6.27 b	34.87 ± 6.04 b

Mean values (numbers before ±) and standard deviations (numbers after ±) were represented (n = 8). Various letters indicate means that the values were significantly differences at $P < 0.05$. To determine the significance of the differences between the results from different treatments was based on one-way variance analysis (ANOVA) combined with the Tukey–Kramer multiple comparison post hoc test. Bold letters indicate the significant differences between treatments. The following elements were below detection limit: As < 0.4 mg kg^{-1}; Cd < 0.02 mg kg^{-1}; Co < 0.04 mg kg^{-1}; Hg < 0.12 mg kg^{-1}; Pb < 0.3 mg kg^{-1}; Se < 0.6 mg kg^{-1}. C: control; WSC: wastewater sludge compost treated; WSC + AMF1: wastewater sludge compost treated and inoculated by AMF1 strain (*Funneliformis mosseae*); WSC + AMF2: wastewater sludge compost and inoculated by AMF2 strain (*F. geosporum*), WSC + AMFmix: wastewater sludge compost treated and inoculated by the mixture of AMF1 and AMF2 (1:1)

and associated microbiota. Molecular studies confirmed a plant growth promoting and a mycorrhiza helper bacteria-rich microbiome associated with AMF which were able to promote both AMF and host plant growth and development (Hao et al. 2021; Ujvári et al. 2021), and those symbiotic relationships are strain-specific (Jäderlund et al. 2008).

Conclusion for future biology

Our research demonstrated that *Arundo donax* L. could be grown on WSC-treated soil. If supplied in moderate quantity, WSC has no noticeable phytotoxic impact on either the growth potential or the physiology of the plants, so WSC could be a good candidate as a nutrient additive for a giant reed. No difference in colonization was observed between each inoculated and the uninoculated plants. However, the addition of selected AMF inoculation might have improved the enzymatic activity of the soil. Further analysis of AMF composition would be required in subsequent experiments to provide evidence of the contribution of the added AMF. In the case of the inoculation by *F. geosporum*, a significant impact on the pattern of the spectra of substrate-induced respiration was observed, especially in breaking down of glutamine and glutamate. MicroResp™, a measurement technique of substrate-induced respiration seemed to be a relevant test to detect the microbial effect of AMF inoculation. Giant reed's invasiveness and tolerance to a variety of environmental conditions may be explained by its efficient regulatory mechanism to adjust optimal/maximal colonization rate and to select for preferential AMF partners. The selection of endophytic fungi that support *A. donax* in various low-fertility or contaminated environments provides an opportunity to establish efficient plant–microbe interactions to improve soil services.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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