

Effects of mycorrhizal inoculation and digestate fertilisation on triticale biomass production using fungicide-coated seeds

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Abstract

Crop fertilisation management using organic wastes and arbuscular mycorrhizal fungi (AMF) inoculation can play a crucial role in the sustainability of agroecosystems. However, in conventional agricultural systems, agrochemicals like fungicides could reduce the positive effect of AMF. The aim of this study was to evaluate the agronomic (biomass production) and environmental (soil CO₂ emission) effects of AMF inoculation and digestate spreading on triticale cultivation using commercial seeds coated with fungicide. The field experiment was conducted in 2014–2015 at the University of Padua's experimental farm (Italy), adopting a split-plot design, where the main plot factor was AMF inoculation (inoculated vs. uninoculated) and the subplot factor was fertilisation treatment (no fertilisation (NF), digestate liquid fraction (DL), digestate solid fraction (DS), mineral fertilisation (MF)). Low AMF root colonization was observed, likely due to the effect of fungicide; the only significant effect of AMF inoculation was a lower shoot density. Dry biomass production was significantly higher in the MF treatment (21.8 ± 1.04 Mg/ha) and lower in the NF treatment (14.5 ± 0.73 Mg/ha) compared to DS and DL treatments, which were not significantly different with an average yield of 17.2 ± 2.10 Mg/ha. During the cropping season, soil CO₂ emissions were not significantly affected by either AMF inoculation or fertilisation treatment. The median value of soil CO₂ emissions was 447.3 mg/m² per hour.

Keywords

arbuscular mycorrhizal fungi • soil CO₂ emission • sterol biosynthesis inhibitor • × Triticosecale sp. Wittmack

Introduction

During the past century, industrialization of agriculture resulted in a significant increase in yield, which led to a greater amount of food available for the population (Pérez-Montano *et al.*, 2014). However, in the last 30 years, the characteristics of climate change, especially precipitation and temperature regimes (Milošević *et al.*, 2015), caused mainly by human activities have led to significantly unstable annual yields in the major crops (maize, soybean, rice and wheat), promoting a negative trend in the global harvest area (Izumi and Ramankutty, 2016; Lesk *et al.*, 2016). Furthermore, food demand, linked to the ever-increasing world population, has increased in the last few decades and is expected to increase in the future. This growing pressure on agroecosystems to maximize their productivity per unit area, time and input (e.g., nutrients, water, energy) (Lal, 2008) can bring environmental impacts such as loss in soil organic matter and fertility and increase in emission of greenhouse gas. In this context, organic fertilisation can be an efficient practice to reduce such impacts (Montemurro *et al.*, 2007). As is well known, soil organic matter plays a significant role in preserving and

improving soil fertility, with positive effects on physical, chemical and biological properties of soil (Montemurro *et al.*, 2004), including soil carbon stocks (Raviv *et al.*, 1998; Caravaca *et al.*, 2002).

The symbiotic association between arbuscular mycorrhizal fungi (AMF) and the roots of many terrestrial plant species is widespread in the natural environment and can provide considerable benefits to the host plant (Gosling *et al.*, 2006; Cavagnaro, 2014; Berruti *et al.*, 2015; Langeroodi *et al.*, 2017). In particular, AMF plays an important role in crop nutrition by greatly increasing the absorptive surface of the root system. Furthermore, they allow to overcome the depletion zones of nutrients around the root system through extraradical mycelium (Tibbett, 2000; Facelli and Facelli, 2002) and to increase uptake of nutrients in an inorganic form, principally immobile phosphate (P) (Koide, 1991; George *et al.*, 1995; Clark and Zeto, 2000) and nutrients from organic sources (Hodge *et al.*, 2001; Hodge and Fitter, 2010). In addition, by influencing soil microorganisms, AMF indirectly influence biochemical reactions of soil including organic matter mineralization and nitrification (Hamel, 2004).

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AMF association provides many ecosystem services such as improving crop nutrient and water uptake and enhancing tolerance and/or resistance to abiotic and biotic stresses (Gosling *et al.*, 2006). In return, the AMF receive carbon (C) from the host plant. Unsuitable agricultural practices affect activities of soil microorganisms; soil tillage, chemical fertilisers (Borriello *et al.*, 2012; Berruti *et al.*, 2014; Verzeaux *et al.*, 2016, 2017) and agrochemical products (Dangi *et al.*, 2017), such as fungicides, used in coated seeds to control pathogens can have undesirable effects on non-target, plant-beneficial microorganisms such as AMF (Campagnac *et al.*, 2008), causing a reduction in species richness and diversity in AMF communities (Gosling *et al.*, 2006). Several authors report higher levels of AMF colonization, higher propagule numbers or higher diversity in organic farming systems (Oehl *et al.*, 2003, 2004; Bending *et al.*, 2004), suggesting that AMF can compensate for the reduced use of mineral fertilisers, especially P (Galvez *et al.*, 2001). However, the actual importance of AMF in the enhancing of ecosystem and agroecosystem's resilience and functions, and in particular crop performance, remains to be determined (Gosling *et al.*, 2006).

The application of high-quality organic materials as a soil amendment and/or fertiliser is the basis to support low-input sustainable agriculture, increasing or preserving soil organic matter content, improving fertility and optimizing crop production (Mäder *et al.*, 2002; Diacono and Montemurro, 2010).

Digestate is the byproduct of anaerobic digestion and, due to process characteristics, it is considered as a good quality soil fertiliser and/or amendment (Nicoletto *et al.*, 2013a, 2013b, 2014; Maucieri *et al.*, 2016). Its chemical composition depends on the feedstock and can therefore vary (Möller and Müller, 2012). The use of digestate (liquid and solid fractions) for soil fertilisation and/or amendment has led to an improvement in soil fertility with a decrease in the amount of chemical fertiliser used in cropping systems (Albuquerque *et al.*, 2012; Möller and Müller, 2012).

Triticale (\times *Triticosecale* sp. Wittmack ex A. Camus 1927) is a hybrid cereal created in the 1960s by a cross between a female parent wheat (*Triticum* spp. L.) and a male parent rye (*Secale cereale* L.). This cross has provided triticale with agronomic features such as the yield of wheat and the hardness of rye. Interest in triticale has steadily increased, and cultivars have been grown in more than 30 countries (McGoverin *et al.*, 2011) as it is a suitable alternative to other cereals (Bassu *et al.*, 2013). In the last 13 years, the total triticale cultivation area in Europe has increased by approximately 45% (FAOSTAT, 2014). On the basis of its agronomic features, triticale is an interesting crop in optimal and marginal cultivation areas of Mediterranean (Ehdaie *et al.*, 2001; Giunta *et al.*, 2003). Currently, it is mainly cultivated for grazing, fresh forage,

silage and hay production. Indeed, cereal straw is a main animal feed source and the use of triticale straw is in continuous expansion, especially in Mediterranean and semi-arid countries (Cazzato *et al.*, 2012).

The aim of this study was to evaluate the agronomic (biomass production) and environmental (soil CO₂ emission) effects of AMF inoculation and digestate spreading on a triticale crop using commercial seeds coated with fungicide.

Materials and methods

Experimental description

The experiment was conducted in the 2014–2015 cropping season at the University of Padua's experimental farm "Lucio Toniolo", North-East Italy (45°20' N; 11°57' E) under field conditions. The climate of the site is subhumid (Köppen climate classification), with mean annual rainfall of approximately 850 mm distributed fairly uniformly throughout the year. The temperature increases from January (average minimum value: -1.5°C) to July (average maximum: 27.2°C). According to the FAO–UNESCO classification, the soil is a fulvi-calcaric Cambisol with a loamy texture. The experimental design adopted for the trial was a split-plot design with three replications. AMF inoculation was the main plot (inoculated (AMF-Y) vs. uninoculated (AMF-N)), whereas fertilisation treatments were the subplots (no fertilisation (NF), mineral fertilisation (MF), digestate liquid fraction (DL) and digestate solid fraction (DS)) randomly distributed. Each plot was 16 m² (4 × 4 m) with a total of 24 plots. MF was applied for three times as follows: i) before sowing: 40 kg N/ha as ammonium nitrate, 65 kg P/ha as superphosphate and 65 kg K/ha as potassium sulfate and ii) top-dressing: 50 kg N/ha as ammonium nitrate at 116 and 173 days after sowing (DAS). Organic fertilisation with DL and DS was applied manually before sowing at a rate equivalent to 140 kg N/ha. Owing to their chemical composition (Table 1), 16 kg P/ha and 88 kg K/ha for DL and 105 kg P/ha and 109 kg K/ha for DS were also applied.

Triticale (cv. Cosinus) was sown on 28 October 2014 at a rate of 220 kg seeds/ha using commercial seeds coated with fungicide (2.34% fludioxonil, 2.34% difenoconazole and 0.93% tebuconazole; Celest® Trio Syngenta AG, Basel, Switzerland). AMF inoculation was done with a commercial inoculum (based on mycorrhizal fungi: *Funneliformis mosseae*, *Funneliformis caledonius*, *Funneliformis coronatus*, *Septoglomus viscosum*; saprophytic fungi: *Trichoderma harzianum* and rhizosphere bacteria: *Pseudomonas fluorescens*, *Bacillus subtilis* and *Agrobacterium radiobacter*; Micosat F wp – CCS – Aosta, Italy), mixing it with seeds before sowing at a dose equal to 1.2 kg/ha. A second inoculation was performed at 149 DAS, distributing 1.6 g of commercial mix inoculum suspended in 10 L of water on each AMF-Y plot.

Root sampling and analysis

Root samples were collected from three randomly selected plants per plot during the growing season on three dates (114, 177 and 223 DAS) with a hand-operated soil probe (5 cm diameter) in the 0–20 cm soil layer. Roots were washed clean of soil with a few drops of Tween 20 and then rinsed several times in tap water. Roots were then cleaned with 10% KOH, stained with 5% ink–vinegar and de-stained in distilled water (Vierheilig *et al.*, 1998). AMF colonization percentages were estimated according to Trouvelot *et al.* (1986) as follows: F% = mycorrhization frequency (percentage of root fragments showing fungal colonization), M% = AMF colonization intensity (percentage of fungi structures on the whole root system), m% = AMF colonization intensity (percentage of fungi structures on colonized root fragments), a% = abundance of arbuscules (percentage of arbuscules presence on root fragments showing fungal colonization) and A% = abundance of arbuscules (percentage of arbuscules presence on the whole root system).

Triticale agronomic measurements

During the growing season, the Normalized Difference Vegetation Index (NDVI) was measured four times from 143 to 178 DAS using an APS1-CropCircle spectrometer (Holland Scientific, Lincoln, NE, USA).

On 8 June 2015, the aerial biomass was harvested (at dough stage (80) BBCH (Biologische Bundesantalt, Bundessortenamt and CHemische Industrie, Germany) scale (Hess *et al.*, 1997)) and dry weight was determined by drying in a thermoventilated oven at 65°C until constant weight was reached. In addition, culm height and shoot density in each plot were determined at harvest.

Soil CO₂ emission

Soil CO₂ emissions were measured in each plot seven times from 78 to 178 DAS. CO₂ flux was measured with the static non-stationary chamber technique (Maucieri *et al.*, 2016) using a chamber with a volume of 5 L and 10 cm square base. Soil CO₂ flux was determined by measuring the temporal change in CO₂ concentration inside the chamber using a portable infrared instrument (Geotech G150), detecting CO₂ concentrations at levels of parts per million.

CO₂ flux (mg CO₂/m² per second) was calculated using the following formula:

$$\text{CO}_2 = \frac{V}{A} \times \frac{dc}{dt}$$

where V (m³) is the volume, A (m²) the footprint of the flux chamber, c the CO₂ concentration (mg CO₂/m³) and t the time step (s).

At each CO₂ measurement point, soil temperature and moisture (FieldScout TDR 100, Spectrum Technologies, Inc.,

Table 1. Main characteristics of the digestate on dry weight basis

Parameter	Liquid fraction	Solid fraction
Dry matter (%)	6.16	24.68
TKN (% DM)	9.09	2.23
NH ₄ -N (% DM)	5.10	0.94
NO ₃ -N (% DM)	0.08	0.03
TP (% DM)	1.02	0.72
K (% DM)	5.41	1.53
Ca (% DM)	1.57	0.70
Mg (% DM)	0.94	0.62
Na (% DM)	0.46	0.12

DM = dry matter, TKN = total Kjeldahl nitrogen, NH₄-N = ammonium nitrogen, NO₃-N = nitrate nitrogen, TP = total phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Na = sodium.

Plainfield, IL, USA) in the first 7.5 cm were also determined. Cumulative CO_{2(eq)} emission saving due to substitution of mineral fertilisers with digestate fractions was calculated according to the quantity of macronutrients supplied (N, P₂O₅, K₂O) and using the specific emission factors reported in Capponi *et al.* (2012). In particular, considering production of mineral fertilisers, the estimated avoided CO_{2(eq)} emissions were 3.26 kg CO_{2(eq)} for each kilogram of N, 2.01 kg CO_{2(eq)} for each kilogram of P₂O₅ and 1.41 kg CO_{2(eq)} for each kilogram of K₂O.

Statistical analysis

The statistical analysis was carried out using the Statistica software (StatSoft Inc., www.statistica.io). AMF colonization percentage values were arcsine transformed before analysis. Aerial biomass' dry weight, culm height, shoot density and AMF colonization percentage values were subjected to a two-way analysis of variance (ANOVA) to assess the interactions between the two fixed factors (AMF inoculation and fertilisation treatments). NDVI values were statistically analyzed using repeated measures ANOVA with DAS, AMF and fertilisation as studied factors. The data were post hoc tested ($P < 0.05$) using the Fisher's LSD test. Agronomic and AMF colonization values are reported as average value \pm standard error.

Since soil CO₂ emission data were not normally distributed, they were analysed with Kruskal–Wallis and Mann–Whitney non-parametric tests. Correlations between CO₂ emissions and both soil temperature and moisture were evaluated using Spearman's rank correlation.

Results

Root mycorrhization

Triticale roots showed no mycorrhizal colonization at 114 and 177 DAS. Instead, at harvest, which was at the hard-dough stage (223 DAS), AMF root colonization was observed without

a significant difference between inoculated and uninoculated plots. AMF colonization values ranged from 5.0% to 85.0% for F%, from 0.1% to 19.2% for M%, from 1.0% to 29.5% for m%, from 0.0% to 60.6% for a% and from 0.0% to 11.6% for A%. Regardless of AMF inoculation, DS treatment showed significantly ($P < 0.05$) higher values for F%, M% and A% compared to the other treatments (Table 2).

Triticale agronomic traits

AMF inoculation had no statistically significant effect on culm height and NDVI, with mean values of 125 ± 6.9 cm (culm height) and 0.64 ± 0.07 (NDVI). AMF inoculation resulted in a significant ($P < 0.05$) decrease in shoot density (-14.2%) compared to the AMF-N treatment (461.3 ± 13.8 culms/m²) (Table 3). Shoot density was significantly ($P < 0.05$) higher in the MF treatment compared to the NF treatment; shoot density in the DS treatment did not differ significantly from the MF treatment. Fertilisation treatment influenced significantly ($P < 0.01$) the NDVI, with the highest values always found for the MF treatment and the lowest for the NF treatment (Table 4). NDVI

was also influenced significantly ($P < 0.01$) by DAS (Table 4). Dry matter yield (DMY) was significantly ($P < 0.01$) higher in the MF treatment with 21.8 ± 1.04 Mg/ha compared to organic fertilisation, which showed, as an average of DS and DL treatments, a decrease of 21.2%. The lowest DMY, as expected, was found in the NF treatment (14.5 ± 0.3 Mg/ha) with a significant ($P < 0.01$) decrease of 33.5% and 15.5% compared to the MF treatment and the mean of DS and DL treatments, respectively (Figure 1). No significant difference ($P = 0.464$) was seen in relation to AMF inoculation, and there was no significant interaction between fertilisation and AMF inoculation ($P = 0.128$) on DMY.

Soil CO₂ emission

No significant difference on soil CO₂ emission was detected among both AMF inoculation and fertilisation treatments (Figure 2), with a median emission value of 447.3 mg/m² per hour. During soil CO₂ emission measurements, moisture in the upper 7.5 cm soil layer ranged from 23.8% to 57.4% and the temperature was from +4.8°C to +16.8°C. On the average of

Table 2. Fertilisation treatment effects on AMF colonization at 223 DAS (mean \pm s.e.)

Mycorrhizal index	Fertilisation				Significance (F)	LSD (P < 0.05)	LSD (P < 0.01)
	MF	DL	DS	NF			
F%	19.2 \pm 5.4	13.3 \pm 2.8	70.0 \pm 7.5	17.5 \pm 4.2	**	12.76	17.71
m%	9.3 \pm 4.3	14.6 \pm 4.1	15.5 \pm 3.7	18.3 \pm 7.8	ns	-	-
M%	2.8 \pm 1.9	1.9 \pm 0.6	11.3 \pm 2.8	3.8 \pm 1.7	*	8.72	-
a%	36.4 \pm 12.4	20.4 \pm 9.5	50.5 \pm 3.2	30.4 \pm 10.0	ns	-	-
A%	1.3 \pm 0.8	0.5 \pm 0.24	6.0 \pm 1.7	1.7 \pm 0.8	*	6.75	-

AMF = arbuscular mycorrhizal fungi, DAS = days after sowing, MF = mineral fertilisation, DL = digestate liquid fraction, DS = digestate solid fraction, NF = no fertilisation, F% = mycorrhization frequency (the percentage of root fragments showing fungal colonization), m% = AMF colonization intensity (the percentage of fungi structures referred to colonized root fragments), ns = not significant, M% = AMF colonization intensity (the percentage of fungi structures referred to the whole root system), a% = abundance of arbuscules (percentage of arbuscules present referred to the root fragments showing fungal colonization), A% = abundance of arbuscules (percentage of arbuscules present referred to the whole root system), * = $P < 0.05$, ** = $P < 0.01$.

Table 3. Effects of AMF inoculation and fertilisation treatments on shoot density (mean \pm s.e.)

Parameter	AMF treatment	Fertilisation				Mean
		MF	DL	DS	NF	
Shoot density (culms/m ²)	AMF-Y	425.3 \pm 7.4	388.0 \pm 13.9	393.3 \pm 16.2	377.3 \pm 33.8	396.0 \pm 10.2
	AMF-N	489.3 \pm 10.9	440.0 \pm 17.4	501.3 \pm 26.9	414.7 \pm 23.1	461.3 \pm 13.8
	Mean	457.3 \pm 15.5	414.0 \pm 15.3	447.3 \pm 27.9	396.0 \pm 20.1	428.6 \pm 10.8
ANOVA		d.f.	Significance (F)		LSD (P < 0.05)	
AMF		1	*		40.66	
Fertilisation		3	*		44.11	
AMF \times fertilisation		3	ns		-	
Residual		12				
Total		23				

AMF = arbuscular mycorrhizal fungi, MF = mineral fertilisation, DL = digestate liquid fraction, DS = digestate solid fraction, NF = no fertilisation, AMF-Y = inoculated treatment, AMF-N = uninoculated treatment, ANOVA = analysis of variance, ns = not significant, * = $P < 0.05$.

Table 4. NDVI at four different DAS times (mean ± s.e.)

DAS	AMF treatment	Fertilisation				Mean
		MF	DL	DS	NF	
143	AMF-Y	0.770±0.022	0.688±0.022	0.688±0.035	0.583±0.020	0.677±0.023
	AMF-N	0.730±0.009	0.695±0.018	0.671±0.022	0.591±0.051	0.672±0.020
	Mean	0.750±0.014	0.691±0.013	0.670±0.018	0.587±0.025	0.675±0.015
153	AMF-Y	0.713±0.010	0.652±0.016	0.651±0.017	0.573±0.021	0.647±0.017
	AMF-N	0.726±0.005	0.666±0.005	0.664±0.012	0.562±0.026	0.655±0.019
	Mean	0.720±0.006	0.659±0.008	0.658±0.010	0.567±0.015	0.651±0.012
162	AMF-Y	0.692±0.009	0.615±0.008	0.612±0.006	0.557±0.013	0.619±0.015
	AMF-N	0.727±0.001	0.605±0.013	0.632±0.012	0.527±0.018	0.623±0.022
	Mean	0.709±0.009	0.610±0.007	0.622±0.008	0.542±0.012	0.621±0.013
178	AMF-Y	0.740±0.013	0.639±0.022	0.637±0.039	0.513±0.015	0.632±0.026
	AMF-N	0.731±0.020	0.589±0.002	0.622±0.026	0.498±0.021	0.610±0.027
	Mean	0.736±0.011	0.614±0.015	0.629±0.021	0.506±0.012	0.621±0.018

ANOVA	d.f.	Significance (F)	LSD (P < 0.05)	LSD (P < 0.01)
DAS	3	**	0.036	0.048
AMF	1	ns	-	-
Fertilisation	3	**	0.021	0.027
DAS × AMF	3	ns	-	-
DAS × fertilisation	9	ns	-	-
AMF × fertilisation	3	ns	-	-
DAS × AMF × fertilisation	9	ns	-	-
Residual	48			
Total	95			

NDVI = Normalized Difference Vegetation Index, DAS = days after sowing, AMF = arbuscular mycorrhizal fungi, MF = mineral fertilisation, DL = digestate liquid fraction, DS = digestate solid fraction, NF = no fertilisation, AMF-Y = inoculated treatment, AMF-N = uninoculated treatment, ANOVA = analysis of variance, ns = not significant, ** = $P < 0.01$.

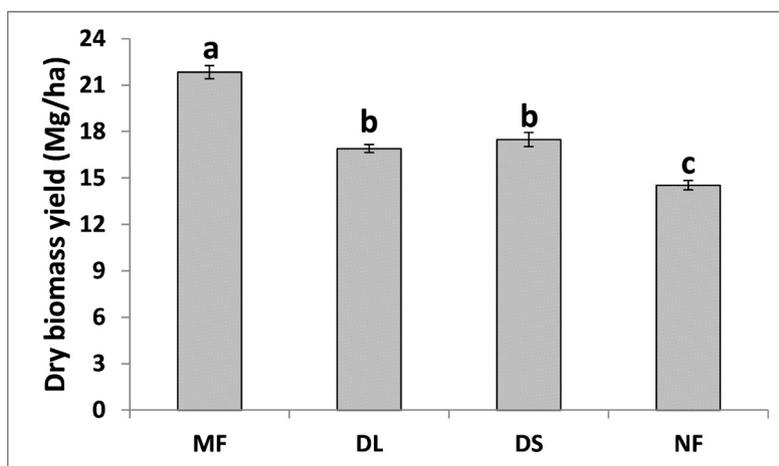


Figure 1. Dry biomass yield under different fertilisation treatments (mean ± s.e.) on the average of AMF inoculation. MF = mineral fertilisation, DL = digestate liquid fraction, DS = digestate solid fraction, NF = no fertilisation. Different letters indicate significant differences among fertilisation treatments for Fisher’s LSD test. (LSD at $P < 0.05$ = 1.09 and LSD at $P < 0.01$ = 1.52).

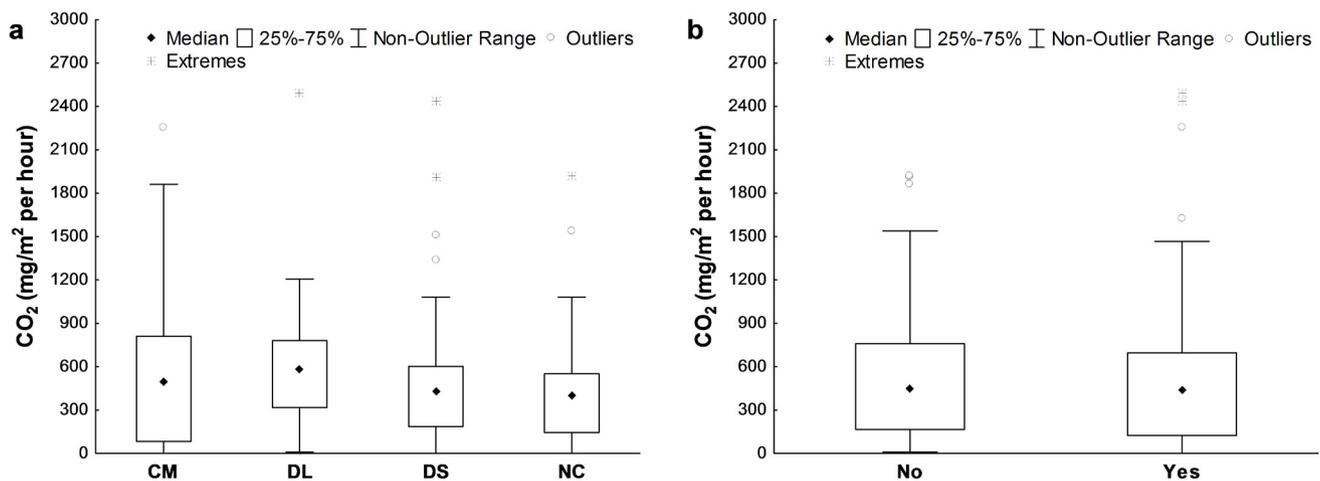


Figure 2. Box-plot diagrams of soil CO₂ emissions in relation to fertilisation (a) and AMF inoculation (b). MF = mineral fertilisation, DL = digestate liquid fraction, DS = digestate solid fraction, NF = no fertilisation, Yes = AMF inoculated and No = AMF uninoculated.

treatments, soil CO₂ emissions were positively correlated with soil temperature (Spearman's $R = 0.617$; $P < 0.001$), whereas no correlations were found with soil moisture (Spearman's $R = -0.015$).

Considering the macronutrients' content of DL and DS (N, P₂O₅, K₂O) treatments and using the CO₂_{2(eq)}-specific emission factors for production of mineral fertilisers (Capponi *et al.*, 2012), the theoretically avoided carbon emission into the atmosphere due to the substitution of mineral fertilisers with nutrients supplied through digestate was -674.4 and -1121.2 kg CO₂_{2(eq)}/ha for DL and DS treatments, respectively. On the contrary, MF treatment determined a net CO₂_{2(eq)} emission to the atmosphere of +863.8 kg CO₂_{2(eq)}/ha.

Discussion

Root mycorrhization

Although the levels of AMF colonization (A%) found in triticale roots were lower than those reported in the literature, within a range of 25%–66% (Pandey *et al.*, 2005, Brito *et al.*, 2012), it could be suggested that AMF inoculation was not effective, possibly due to the fungicide seed treatment (Celest® Trio) consisting of fludioxonil and two sterol biosynthesis inhibitors (SBIs), difenoconazole and tebuconazole. When used alone, fludioxonil does not seem to affect AMF activity, as reported by Murillo-Williams and Pedersen (2008) who observed a better AMF root colonization on soybean seed where a fungicide (fludioxonil) was applied, potentially due to lower competition by aggressive pathogens. However, if fludioxonil is used together with systemic fungicides, it can have a negative effect on AMF colonization (Jin *et al.*, 2013). Indeed, these latter authors found a reduction (-9.4%) of mycorrhizal colonization

in pea by indigenous AMF in response to the application of fungicides with systemic and non-systemic activities (metalaxyl and fludioxonil). Interestingly, this suppressive effect on mycorrhizal colonization was more pronounced in inoculated plants. Moreover, the same authors found that the suppressive effect was present only when commercial AMF inoculum was used in chickpea (-15.6%). Campagnac *et al.* (2008), Zocco *et al.* (2008) and Calonne *et al.* (2010), using various SBI fungicides at different concentrations on root organ cultures (ROCs), reported a drastic reduction in AMF life cycle (spore germination, germ tube elongation, percentage of colonization, extra-radical hyphal growth and sporulation) of *Rhizophagus intraradices* and *Rhizophagus irregularis*. Therefore, in our study, the low AMF root colonization could have been caused by the mechanism of action of difenoconazole and tebuconazole. Considering the previous years' conventional farm management with the use of fungicides, the low root colonization could be due to the detrimental effect exerted by these molecules on persistence of the native AMF community in the soil.

Moreover, several authors have demonstrated that the application of high amounts of chemical fertilisers (particularly P and N) in intensive agricultural systems to improve crop yield negatively affects AMF root colonization and the number of AMF propagules in the soil (Johnson, 1993; Liu *et al.*, 2000; Kahiluoto *et al.*, 2001; Burrows and Pflieger, 2002; Treseder and Allen, 2002). On the contrary, organic sources of nutrients (manure, compost and crop residues) and slow-release fertilisers (such as rock phosphates) stimulate AMF activity (Gosling *et al.*, 2006). A proportion of the phosphorus contained in DS is in the form of struvite (formed during the anaerobic digestion process) (Möller and Müller, 2012), a low available P for uptake by plants that may have contributed to the higher

AMF colonization in that treatment. In fact, Heydari and Maleki (2014) observed significantly higher AMF colonization levels in inoculated barley supplied with rock phosphate and struvite compared to other fertilisation treatments. In addition, the increased amount of organic matter present in the soil due to the DS supply could have reduced the effects of the hydrophobic fungicides (difenoconazole and tebuconazole) on AMF activity. This is supported by the paper of Roy *et al.* (2000) who observed an improved sorption of hydrophobic fungicides by humic substances in the presence of low soil moisture.

Triticale agronomic traits

Shoot density was significantly higher in the absence of AMF inoculation, which is in agreement with Hartnett *et al.* (1994) who found a similar response for other *Poaceae* species. Despite AMF inoculation, the highest DMY was obtained in MF treatment and the lowest in NF treatment. Similar triticale DMY, using mineral fertilisers in a Mediterranean area, was reported by Santiveri *et al.* (2004) (24.3 Mg DMY/ha with 92 kg N/ha) and Giunta and Motzo (2004) (22.3 Mg DMY/ha with 97 kg N/ha), whereas, in the same cultivation area of our study (Po Valley), Delogu *et al.* (2002), supplying 170 kg N/ha, reported a DMY at milk-dough stage from 12.3 to 17.2 Mg/ha depending on cultivars. Although our study covered only 1 year, the yield comparability with the average of literature data supports the reliability of our findings. The lower DMY of the digestate treatments was probably due to the lower efficacy of N, since the other two macronutrients, P and K, applied with MF were lower compared to DS and lower (P) and higher (K) compared to DL. The lower efficacy of N applied with digestate can be due to: i) the distribution period (all at pre-sowing in DL and DS treatments, at three times in MF treatment) and ii) possible ammonia volatilization losses (on average 15% $\text{NH}_4^+\text{-N}$ applied) that occur mainly within the first 10 h after digestate distribution (Quakernack *et al.*, 2012), especially in DL treatment where $\text{NH}_4^+\text{-N}$ represents ~56% of total Kjeldahl nitrogen.

Soil CO₂ emission

The finding that there was no significant difference in soil CO₂ emission among fertilisation treatments is in agreement with our previous research (Maucieri *et al.*, 2016), where we observed, using only DL treatment, a significant increase in soil CO₂ emission, only in the first days after distribution. The lack of significant differences in soil CO₂ emission among fertilisation treatments can be attributed to the characteristics of organic matter content in the digestate. The digestate used in this experiment came from a mesophilic (35–40°C) anaerobic digestion plant that had a substrate retention time of 88–92 days. According to Maucieri *et al.* (2017), we can assume that, except for the low residual quantity of easily available organic matter mostly degradable in the short term

(Albuquerque *et al.*, 2012), stabilized organic matter was supplied, which did not influence soil CO₂ emission during the monitored period.

Conclusions

To our knowledge, this is the first field study on triticale biomass production that evaluates the combined effects of AMF inoculation and digestate fertilisation using seeds coated with fungicide.

The obtained results indicate that AMF inoculation resulted in a reduction of shoot density without any significant effect on biomass production. All other parameters were not significantly affected by AMF inoculation.

On the basis of biomass production, although lower than that obtained using chemical fertilisers, triticale fertilisation with digestate could be an interesting agronomic practice in sustainable agriculture to reduce environmental and economic costs.

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