Research Article • DOI: 10.1515/ijafr-2018-0005 IJAFR • 57 • 2018 • 42-51

Irish Journal of Agricultural and Food Research

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

C. Caruso[1], C. Maucieri[1†], A. Barco[1], A.C. Barbera[2,3], M. Borin[1]

[1]Department of Agronomy, Food, Natural Resources, Animals and Environment – DAFNAE, University of Padua, Agripolis Campus, viale dell'Università 16, 35020 Legnaro, PD, Italy
[2]Department of Agriculture, Food and Environment – Di3A, University of Catania, Via Valdisavoia 5, 95123 Catania, CT, Italy
[3]Associated Researcher, Trees and Timber Institute, National Research Council of Italy (CNR-IVALSA), Via Paolo Gaifami 18, Catania, CT, Italy

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 CO_2 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 CO_2 emissions were not significantly affected by either AMF inoculation or fertilisation treatment. The median value of soil CO_2 emissions was 447.3 mg/m² per hour.

Keywords

arbuscular mycorrhizal fungi • soil CO_2 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 (Iizumi 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).

†Corresponding author: Carmelo Maucieri
E-mail: carmelo.maucieri@unipd.it

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 (Alburquerque *et al*., 2012; Möller and Müller, 2012).

Triticale (× *Triticosecale* sp. Wittmack ex A. Camus 1927) is an 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 hardiness 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 CO_2 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 m^2 (4 × 4m) 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 CO_2 emission

Soil CO_2 emissions were measured in each plot seven times from 78 to 178 DAS. CO_2 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 CO_2 flux was determined by measuring the temporal change in CO_2 concentration inside the chamber using a portable infrared instrument (Geotech G150), detecting CO_2 concentrations at levels of parts per million.

CO_2 flux (mg CO_2/m^2 per second) was calculated using the following formula:

$$CO_2 = \frac{V}{A} \times \frac{dc}{dt}$$

where V (m^3) is the volume, A (m^2) the footprint of the flux chamber, c the CO_2 concentration (mg CO_2/m^3) and t the time step (s).

At each CO_2 measurement point, soil temperature and moisture (FieldScout TDR 100, Spectrum Technologies, Inc., Plainfield, IL, USA) in the first 7.5 cm were also determined.

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, NH_4-N = ammonium nitrogen, NO_3-N = nitrate nitrogen, TP = total phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Na = sodium.

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, P_2O_5, K_2O) 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 P_2O_5 and 1.41 kg $CO_{2(eq)}$ for each kilogram of K_2O.

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 ± standard error.

Since soil CO_2 emission data were not normally distributed, they were analysed with Kruskal–Wallis and Mann–Whitney non-parametric tests. Correlations between CO_2 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/m^2) (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 CO_2 emission

No significant difference on soil CO_2 emission was detected among both AMF inoculation and fertilisation treatments (Figure 2), with a median emission value of 447.3 mg/m^2 per hour. During soil CO_2 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 ± s.e.)

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

Parameter	AMF treatment	Fertilisation				Mean
		MF	DL	DS	NF	
Shoot density (culms/m^2)	AMF-Y	425.3 ± 7.4	388.0 ± 13.9	393.3 ± 16.2	377.3 ± 33.8	396.0 ± 10.2
	AMF-N	489.3 ± 10.9	440.0 ± 17.4	501.3 ± 26.9	414.7 ± 23.1	461.3 ± 13.8
	Mean	457.3 ± 15.5	414.0 ± 15.3	447.3 ± 27.9	396.0 ± 20.1	428.6 ± 10.8
ANOVA		**d.f.**	**Significance (F)**		**LSD (P < 0.05)**	
AMF		1	*		40.66	
Fertilisation		3	*		44.11	
AMF × 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, ** = $P < 0.01$.

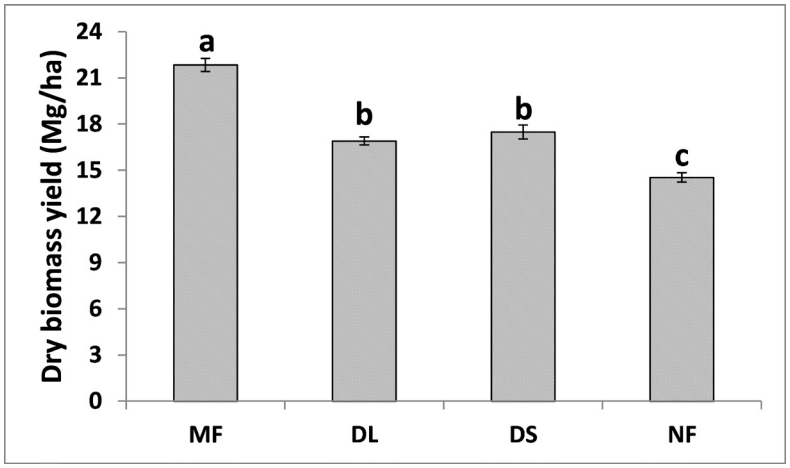

Figure 1. Dry biomass yield under different fertilisation treatments (mean ± s.e.) on the average of AMF inoculation. MF = mineral fertilisation, DL = digestate liquid fraction, DS = digestate solid fraction, NF = no fertilisation. Different letters indicate significant differences among fertilisation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

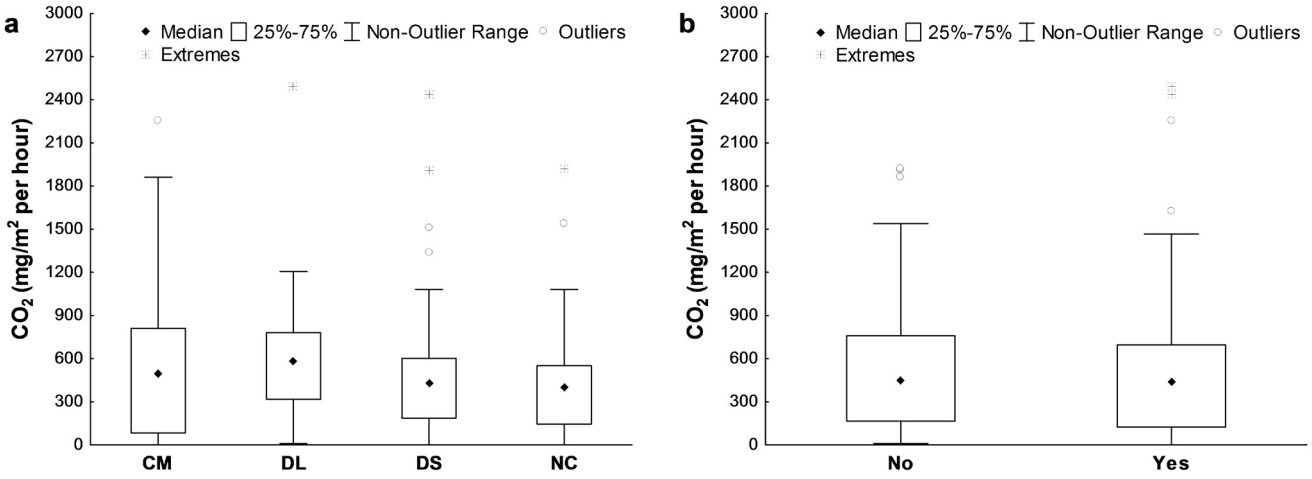

Figure 2. Box-plot diagrams of soil CO_2 emissions in relation to fertilisation (a) and AMF inoculation (b). MF = mineral fertilisation, DL = digestate liquid fraction, DS = digestate solid fraction, NF = no fertilisation, Yes = AMF inoculated and No = AMF uninoculated.

treatments, soil CO_2 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, P_2O_5, K_2O) treatments and using the $CO_{2(eq)}$-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 $CO_{2(eq)}$/ha for DL and DS treatments, respectively. On the contrary, MF treatment determined a net $CO_{2(eq)}$ emission to the atmosphere of +863.8 kg $CO_{2(eq)}$/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 Pfleger, 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% NH_4^+–N applied) that occur manly within the first 10 h after digestate distribution (Quakernack *et al*., 2012), especially in DL treatment where NH_4^+–N represents ~56% of total Kjeldahl nitrogen.

Soil CO_2 emission

The finding that there was no significant difference in soil CO_2 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 CO_2 emission, only in the first days after distribution. The lack of significant differences in soil CO_2 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 (Alburquerque *et al*., 2012), stabilized organic matter was supplied, which did not influence soil CO_2 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.

Acknowledgements

The research was supported by ValDige project "Valorizzazione del digestato per la riduzione delle perdite di CO_2" DGR no. 1604 del 31/07/2012 financed by PSR Regione Veneto (2007–2014) misura 124, Domanda n. 2307827.

References

Alburquerque, J.A., de la Fuente, C., Campoy, M., Carrasco, L., Nájera, I., Baixauli, C., Caravaca, F., Roldán, A., Cegarra, J. and Bernal M.P. 2012. Agricultural use of digestate for horticultural crop production and improvement of soil properties. *European Journal of Agronomy* **43**: 119–128.

Bassu, S., Asseng, S., Giunta, F. and Motzo, R. 2013. Optimizing triticale sowing densities across the Mediterranean Basin. *Field Crops Research* **144**: 167–178.

Bending, G.D., Turner, M.K., Rayns, F., Marx, M.C. and Wood, M. 2004. Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. *Soil Biology and Biochemistry* **36**: 1785–1792.

Berruti, A., Borriello, R., Orgiazzi, A., Barbera, A.C., Lumini, E. and Bianciotto, V. 2014. "Arbuscular Mycorrhizal Fungi and their Value for Ecosystem Management. Biodiversity: The Dynamic Balance of the Planet". InTech, Rijeta, Croacia, pages 159–191.

Berruti, A., Lumini, E., Balestrini, R. and Bianciotto, V. 2015. Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes. *Frontiers in Microbiology* **6**: 1559.

Borriello, R., Lumini, E., Girlanda, M., Bonfante, P. and Bianciotto, V. 2012. Effects of different management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular approach. *Biology and Fertility of Soils* **48**: 911–922.

Brito, I., Goss, M.J. and De Carvalho, M. 2012. Effect of tillage and crop on arbuscular mycorrhiza colonization of winter wheat and triticale under Mediterranean conditions. *Soil Use and Management* **28**: 202–208.

Burrows, R.L. and Pfleger, F.L. 2002. Arbuscular mycorrhizal fungi respond to increasing plant diversity. *Canadian Journal of Botany* **80**: 120–130.

Calonne, M., Fontaine, J., Debiane, D., Laruelle, F., Grandmougin, A. and Lounes-Hadj, S.A. 2010. Side effects of the sterol biosynthesis inhibitor fungicide, propiconazole, on a beneficial arbuscular mycorrhizal fungus. *Communications in Agricultural and Applied Biological Sciences* **76**: 891–902.

Campagnac, E., Fontaine, J., Sahraoui, A.L.H., Laruelle, F., Durand, R. and Grandmougin-Ferjani, A. 2008. Differential effects of fenpropimorph and fenhexamid, two sterol biosynthesis inhibitor fungicides, on arbuscular mycorrhizal development and sterol metabolism in carrot roots. *Phytochemistry* **69**: 2912–2919.

Capponi, S., Fazio, S. and Barbanti, L. 2012. CO_2 savings affect the break-even distance of feedstock supply and digestate placement in biogas production. *Renewable Energy* **37**: 45–52.

Caravaca, F., Hernandez, T., Garcıa, C. and Roldan, A. 2002. Improvement of rhizosphere aggregate stability of afforested semiarid plant species subjected to mycorrhizal inoculation and compost addition. *Geoderma* **108**: 133–144.

Cavagnaro, T.R. 2014. Impacts of compost application on the formation and functioning of arbuscular mycorrhizas. *Soil Biology and Biochemistry* **78**: 38–44.

Cazzato, E., Laudadio, V. and Tufarelli, V. 2012. Effects of harvest period, nitrogen fertilization and mycorrhizal fungus inoculation on triticale (× *Triticosecale* Wittmack) forage yield and quality. *Renewable Agriculture and Food Systems* **27**: 278–286.

Clark, R.B. and Zeto, S.K. 2000. Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition* **23**: 867–902.

Dangi, S.R., Tirado-Corbalá, R., Gerik, J. and Hanson, B.D. 2017. Effect of long-term continuous fumigation on soil microbial communities. *Agronomy* **7**: 37.

Delogu, G., Faccini, N., Faccioli, P., Reggiani, F., Lendini, M., Berardo, N. and Odoardi, M. 2002. Dry matter yield and quality evaluation at two phenological stages of forage triticale grown in the Po Valley and Sardinia, Italy. *Field Crops Research* **74**: 207–215.

Diacono, M. and Montemurro, F. 2010. Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development* **30**: 401–422.

Ehdaie, B., Shakiba, M.R. and Waines, J.G. 2001. Sowing date and nitrogen input influence nitrogen-use efficiency in spring bread and durum wheat genotypes. *Journal of Plant Nutrition* **24**: 899–919.

Facelli, E. and Facelli, J.M. 2002. Soil phosphorus heterogeneity and mycorrhizal symbiosis regulate plant intra-specific competition and size distribution. *Oecologia* **133**: 54–61.

Food and Agriculture Organization of the United Nations. 2014. "FAOSTAT Database". http://faostat3.fao.org.

Galvez, L., Douds, D.D. Jr., Drinkwater, L.E. and Wagoner, P. 2001. Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. *Plant and Soil* **228**: 299–308.

George, E., Marschner, H. and Jakobsen, I. 1995. Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology* **15**: 257–270.

Giunta, F. and Motzo, R. 2004. Sowing rate and cultivar affect total biomass and grain yield of spring triticale (× *Triticosecale* Wittmack) grown in a Mediterranean-type environment. *Field Crops Research* **87**: 179–193.

Giunta, F., Motzo, R. and Pruneddu, G. 2003. Comparison of temperate cereals and grain legumes in a Mediterranean environment. *Agricoltura Mediterranea* **133**: 234–248.

Gosling, P., Hodge, A., Goodlass, G. and Bending, G.D. 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems & Environment* **113**: 17–35.

Hamel, C. 2004. Impact of arbuscular mycorrhizal fungi on N and P cycling in the root zone. *Canadian Journal of Soil Science* **84**: 383–395.

Hartnett, D.C., Samenus, R.J., Fischer, L.E. and Hetrick, B.A.D. 1994. Plant demographic responses to mycorrhizal symbiosis in tallgrass prairie. *Oecologia* **99**: 21–26.

Hess, M., Barralis, G., Bleiholder, H., Buhr, L., Eggers, T.H., Hack, H. and Stauss R. 1997. Use of the extended BBCH scale—general for the descriptions of the growth stages of mono- and dicotyledonous weed species. *Weed Research* **37**: 433–441.

Heydari, M.M. and Maleki, A. 2014. Effect of phosphorus sources and mycorrhizal inoculation on root colonization and phosphorus uptake of barley (*Hordeum vulgare* L.). *International Journal of Current Microbiology and Applied Sciences* **3**: 235–248.

Hodge, A., Campbell, C.D. and Fitter, A.H. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* **413**: 297–299.

Hodge, A. and Fitter, A.H. 2010. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proceedings of the National Academy of Sciences* **107**: 13754–13759.

Iizumi, T. and Ramankutty, N. 2016. Changes in yield variability of major crops for 1981–2010 explained by climate change. *Environmental Research Letters* **11**: 034003.

Jin, H., Germida, J.J. and Walley, F.L. 2013. Suppressive effects of seed-applied fungicides on arbuscular mycorrhizal fungi (AMF) differ with fungicide mode of action and AMF species. *Applied Soil Ecology* **72**: 22–30.

Johnson, N.C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications* **3**: 749–757.

Kahiluoto, H., Ketoja, E., Vestberg, M. and Saarela, I. 2001. Promotion of AM utilization through reduced P fertilization 2. Field studies. *Plant and Soil* **231**: 65–79.

Koide, R.T. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist* **117**: 365–386.

Lal, R. 2008. Soils and sustainable agriculture. A review. *Agronomy for Sustainable Development* **28**: 57–64.

Langeroodi, A.R.S., Ghooshchi, F. and Dadgar, T. 2017. Alleviatory activities in mycorrhizal tobacco plants subjected to increasing chloride in irrigation water. *Italian Journal of Agronomy* **12**: 8–16.

Lesk, C., Rowhani, P. and Ramankutty, N. 2016. Influence of extreme weather disasters on global crop production. *Nature* **529**: 84–87.

Liu, A., Hamel, C., Hamilton, R.I., Ma, B.L. and Smith, D.L. 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* **9**: 331–336.

Mäder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P. and Niggli, U. 2002. Soil fertility and biodiversity in organic farming. *Science* **296**: 1694–1697.

Maucieri, C., Barbera, A.C. and Borin, M. 2016. Effect of injection depth of digestate liquid fraction on soil carbon dioxide emission and maize biomass production. *Italian Journal of Agronomy* **11**: 6–11.

Maucieri, C., Nicoletto, C., Caruso, C., Sambo, P. and Borin, M. 2017. Effects of digestate solid fraction fertilization on yield and soil CO_2 emission in a horticulture succession. *Italian Journal of Agronomy* **12**: 116–123.

McGoverin, C.M., Snyders, F., Muller, N., Botes, W., Fox, G. and Manley, M. 2011. A review of triticale uses and the effect of growth environment on grain quality. *Journal of the Science of Food and Agriculture* **91**: 1155–1165.

Milošević, D., Savić, S.M., Stojanović, V. and Popov-Raljić, J. 2015. Effects of precipitation and temperatures on crop yield variability in Vojvodina (Serbia). *Italian Journal of Agrometeorology* **20**: 35–46.

Möller, K. and Müller, T. 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. *Engineering in Life Sciences* **12**: 242–257.

Montemurro, F., Convertini, G. and Ferri, D. 2004. Mill wastewater and olive pomace compost as amendments for rye-grass. *Agronomie* **24**: 481–486.

Montemurro, F., Maiorana, M., Convertini, G. and Ferri, D. 2007. Alternative sugar beet production using shallow tillage and municipal solid waste fertiliser. *Agronomy for Sustainable Development* **27**: 129–137.

Murillo-Williams, A. and Pedersen, P. 2008. Arbuscular mycorrhizal colonization response to three seed-applied fungicides. *Agronomy Journal* **100**: 795–800.

Nicoletto, C., Santagata, S. and Sambo, P. 2013a. Effect of compost application on qualitative traits in cabbage. *Acta Horticulturae* **1005**: 389–395.

Nicoletto, C., Santagata, S. and Sambo, P. 2013b. Effect of anaerobic digestates application on qualitative traits in early and late cauliflower. *Acta Horticulturae* **1005**: 463–469.

Nicoletto, C., Santagata, S., Zanin, G. and Sambo, P. 2014. Effect of the anaerobic digestion residues use on lettuce yield and quality. *Scientia Horticulturae* **180**: 207–213.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T. and Wiemken, A. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology* **69**: 2816–2824.

Oehl, F., Sieverding, E., Mäder, P., Dubois, D., Ineichen, K., Boller, T. and Wiemken, A. 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia* **138**: 574–583.

Pandey, R., Singh, B. and Nair, T.V.R. 2005. Impact of arbuscular-mycorrhizal fungi on phosphorus efficiency of wheat, rye, and triticale. *Journal of Plant Nutrition* **28**: 1867–1876.

Pérez-Montano, F., Alías-Villegas, C., Bellogín, R.A., del Cerro, P., Espuny, M.R., Jiménez-Guerrero, I., López-Baena, F.J., Ollero, F.J. and Cubo, T. 2014. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. *Microbiological Research* **169**: 325–336.

Quakernack, R., Pacholski, A., Techow, A., Herrmann, A., Taube, F. and Kage, H. 2012. Ammonia volatilization and yield response of energy crops after fertilization with biogas residues in a coastal marsh of Northern Germany. *Agriculture, Ecosystems & Environment* **160**: 66–74.

Raviv, M., Zaidman, B.Z. and Kapulnik, Y. 1998. The use of compost as a peat substitute for organic vegetable transplants production. *Compost Science & Utilization* **6**: 46–52.

Roy, C., Gaillardon, P. and Montfort, F. 2000. The effect of soil moisture content on the sorption of five sterol biosynthesis inhibiting fungicides as a function of their physicochemical properties. *Pest Management Science* **56**: 795–803.

Santiveri, F., Royo, C. and Romagosa, I. 2004. Growth and yield responses of spring and winter triticale cultivated under Mediterranean conditions. *European Journal of Agronomy*, **20**: 281–292.

Tibbett, M. 2000. Roots, foraging and the exploitation of soil nutrient patches: the role of mycorrhizal symbiosis. *Functional Ecology* **14**: 397–399.

Treseder, K.K. and Allen, M.F. 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist* **155**: 507–515.

Trouvelot, A., Kough, J. and Gianinazzi-Pearson, V. 1986. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: "Physiological and Genetical Aspects of Mycorrhizae". (eds. V. Gianinazzi-Pearson and S. Gianinazzi), INRA Press, Paris, pages 217–221.

Verzeaux, J., Nivelle, E., Roger, D., Hirel, B., Dubois, F. and Tetu, T. 2017. Spore density of arbuscular mycorrhizal fungi is fostered by six years of a no-till system and is correlated with environmental parameters in a silty loam soil. *Agronomy* **7**: 38.

Verzeaux, J., Roger, D., Lacoux, J., Nivelle, E., Adam, C., Habbib, H., Hirel, B., Dubois, F. and Tetu T. 2016. In winter wheat, no-till increases mycorrhizal colonization thus reducing the need for nitrogen fertilization. *Agronomy* **6**: 38.

Vierheilig, H., Coughlan, A.P., Wyss, U. and Piché, Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* **64**: 5004–5007.

Zocco, D., Fontaine, J., Lozanova, E., Renard, L., Bivort, C., Durand, R., Grandmougin-Ferjani, A. and Declerck, S. 2008. Effects of two sterol biosynthesis inhibitor fungicides (fenpropimorph and fenhexamid) on the development of an arbuscular mycorrhizal fungus. *Mycological Research* **112**: 592–601.