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Agronomic management of AMF functional diversity to overcome biotic and abiotic stresses - The role of plant sequence and intact extraradical mycelium

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ABSTRACT

Intentional use of arbuscular mycorrhizal fungi (AMF) in cropping systems has been marginal, owing to the high cost and limited biodiversity of commercial inocula, together with the timeliness of colonization to achieve benefits. Additionally, mycorrhiza are considered incompatible with high input cropping systems. Combining results from 4 different experiments resulted in a strategy for the earlier and faster colonization by AMF, through an extensive extraradical mycelium (ERM) acting as a preferential source of inoculum if kept intact by the adoption of appropriate tillage techniques. Selection of host plants on which the ERM develops, provides the tool to manage AMF functional diversity. This strategy resulted in protection of sensitive crop species against biotic and abiotic stresses and can be implemented in low- and high-input cropping systems. Under Mn toxicity arbuscular colonization increased 2.6-fold and shoot dry weight 2.3-fold. In presence of *Fusarium*, arbuscular colonization increased 2.1-fold and shoot dry weight 1.5-fold.

1. Introduction

In the context of the continuing rapid increase in population, food production is probably one of the greatest challenges facing the world (FAO et al., 2017), especially when it needs to be undertaken with reduced environmental impacts on soil, water and air. The adoption of sustainable intensification, with an increase in yield but a reduced level of inputs, is one answer to this dilemma. The required increased efficiency in the use of production factors, such as water, fertilizer, pesticide and energy, provides a challenge to creation of the appropriate scientific and technological advances. The interaction between beneficial soil microbes and plants can play a major role regarding sustainable intensification. Arbuscular mycorrhizal fungi (AMF) can benefit plants in numerous ways. For example they can enhance nutrient and water acquisition, protect against biotic and abiotic stresses, as well as improve soil structure (Smith and Smith, 2012). However, their intentional use in agricultural systems has been marginal, for several reasons:

(1) most available commercial inocula are expensive as a result of the obligatory nature of the symbioses and the associated complexity of their production. Additionally commercial inocula are usually composed of only a few AMF species or isolates; essentially those that are easily multiplied, and therefore their biodiversity is constrained. There is a considerable functional diversity among AMF and, depending on the host plant or the prevailing environmental condition, the symbiosis has different effects (van der Heijden et al., 2004; Antunes et al., 2011). Commercial inocula are, therefore, far from accomplishing the potential benefits offered by AMF in each situation.

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(2) The most benefit from formation of arbuscular mycorrhiza (AM) would be expected from a very diverse indigenous AMF population, well adapted to the specific edaphic and climatic circumstances, and encompassing great functional diversity. Consequently, management of the functional diversity of AMF within the cropping systems would seem to be a more favorable option than inoculation for taking advantage of AMF benefits. However, current understanding of AMF ecology under cropping systems is very limited and the tools for its intentional and strategic management are scarcely a reality.
(3) Before a host plant can gain the benefits of mycorrhiza formation, colonization must be well established and consequently the timeliness can be more crucial than the extent of colonization (Goss and de Varennes, 2002; Khaosaad et al., 2007).

Among the different forms of AMF inoculum, an intact extraradical mycelium (ERM) promotes earlier and faster colonization than others (Fairchild and Miller, 1990; Goss and de Varennes, 2002; Brito et al., 2013). This might be a consequence of AMF fungal abundance together with the fact that intact mycorrhizal networks can colonize a host plant much faster than other inoculum sources (Read et al., 1976). Additionally, the biochemical recognition dialogue between AMF and the host plant might also be involved, as it seems to depend on the inoculum source and involves less signalling from the host plant (David-Schwartz et al., 2001) or a different response by the fungal hyphae depending on the propagule type (Hata et al., 2010). Plant defence mechanisms, both locally and systemically are activated by AMF colonization (Khaosaad et al., 2007; Cameron et al., 2013), therefore, the earlier colonization of the host plant by intact ERM also offers the possibility of precocious activation of those mechanisms. The ERM accompanying microbes, namely plant growth promoting rhizobacteria, can also establish synergistic interactions that can enhance plant growth under normal (non-stress) or stressed environments (Nadeem et al., 2014).

Overall, colonization started by an intact ERM results in an optimization of the potential benefits from AM for the host plant. This is particularly important if the plant is challenged by biotic or abiotic stresses, as the extent of AMF colonization when the host plant comes into contact with the stressor agent is directly related to the level of bioprotection achieved (Sikora et al., 2008). For example, to control onion white rot (*Sclerotium cepivorum*), Torres-Barragán et al. (1996) showed that positive effects were only found when the onions were inoculated with the AMF at the nursery stage, before the plants made contact with the disease organism. Also the prior-inoculation of perennial species in the nursery can enhance bioprotection of species, such as prunes (Cordier et al., 1996), vine (*Vitis rupestris*) (Petit and Gubler, 2006) and olive (*Olea europaea*) (Castillo et al., 2006; Kapulnik et al., 2010). However, such an approach is impossible for crops that are seeded into the field. Furthermore, even for transplanted crops, it requires the use of cultured AMF inoculum, which is likely less diverse and not as well-adapted to local conditions compared with indigenous AMF.

A considerable functional diversity among AMF has long been recognized in terms of colonization rate, development and extensiveness of ERM, efficiency of phosphorus (P) uptake, mycorrhizal-specific gene expression in the host plant (Johnson et al., 1997; van der Heijden et al., 1998; Courty et al., 2015) and protection against biotic and abiotic stresses (Kothari et al., 1991; Thygesen et al., 2004; Lax et al., 2011; Brito et al., 2014). These traits can differ between AMF or even isolates of the same species (Munkvold et al., 2004). Additionally, there may be functional complementarily among AMF species (Wehner et al., 2010). The preference of host plants for specific AM fungal genotypes (Scheublin et al., 2004; Bever et al., 2009; Öpik et al., 2009; Lekberg et al., 2013) is also widely documented, as is the fact that a single host plant can harbour several AMF species or isolates simultaneously (van Tuinen et al., 1998; Brígido et al., 2017). Overall, the expression of AMF functional diversity depends on the AMF species or isolates, the host plant and also a number of environmental factors like climatic conditions or soil type (Smith and Smith, 2012). Consequently, the relationship between taxonomic and functional diversity of AMF and plant productivity is ultimately site specific and its management remains a challenge.

In this paper we provide an evidence-based approach on how the timely colonization allowed by the presence of an intact pre-established mycorrhizal network, together with the strategic management of functional diversity of indigenous AMF, can overcome the major constrains of intentional use of AMF within cropping systems. The research developed in recent years by the authors provides a rationale for increasing the role of mycorrhiza in the development of a sustainable intensification of agricultural systems in different parts of the world.

2. Material and methods

2.1. Protection against abiotic stress

The ability of intact ERM to promote earlier and faster colonization and greater efficacy in protecting wheat (Experiment 1, for details see Brito et al., 2014) and subterranean clover (Experiment 2, for details see Alho et al., 2015) against an abiotic stress were investigated in a two phase pot experiment. Field soil used was unsterilized and contained excessive levels of Mn that impaired growth of both wheat and subterranean clover. The experiments were organized in a randomized block design with fourfold replication and conducted in a greenhouse with temperature control (details of the experimental conditions are described in Brito et al., 2014 and Alho et al., 2015).

In Phase 1 of Experiments 1 and 2, four plant species, which occurred naturally in the ecosystem, were grown, two (*Silene gallica, Rumex bucephalophorus*) being non or scarcely mycotrophic (negative control) and two (*Lolium rigidum* and *Ornithopus compressus*) readily formed mycorrhiza. All these plants were designated as 'Developers' of ERM. An additional negative control with no Developer plants was also included. In both Experiment 1 and 2 all the Developer plants were killed with a systemic herbicide at the end of Phase 1 to ensure that the means of terminating this stage was not a factor in the experiments. In Phase 2 of both experiments, two levels of integrity of the ERM were induced by mechanical disturbance of the soil. In half of the pots the soil was passed through a 4 mm sieve (the Disturbed treatment), which fragmented the ERM developed by any mycorrhiza associated with the Developers. In the remaining pots, there was no mechanical disturbance (the Undisturbed treatment), which kept intact any ERM formed by mycorrhiza on the Developers. Wheat (Experiment 1) or subterranean clover (Experiment 2) seedlings were then planted in each pot. Therefore, at the time when either crop was growing, indigenous AMF were always present but colonization was initiated by different types of propagule. When no mycotrophic Developer plants were grown in Phase 1 of both experiments, AMF spores were the main source of propagules in the soil at the beginning of Phase2. After mycotrophic Developers the AMF propagule types at the beginning of Phase 2 of the experiments were spores, fragmented mycelium and colonised root fragments in the Disturbed soil treatments, and spores and intact ERM in the Undisturbed soil treatments. A schematic representation of Experiments 1 and 2 is

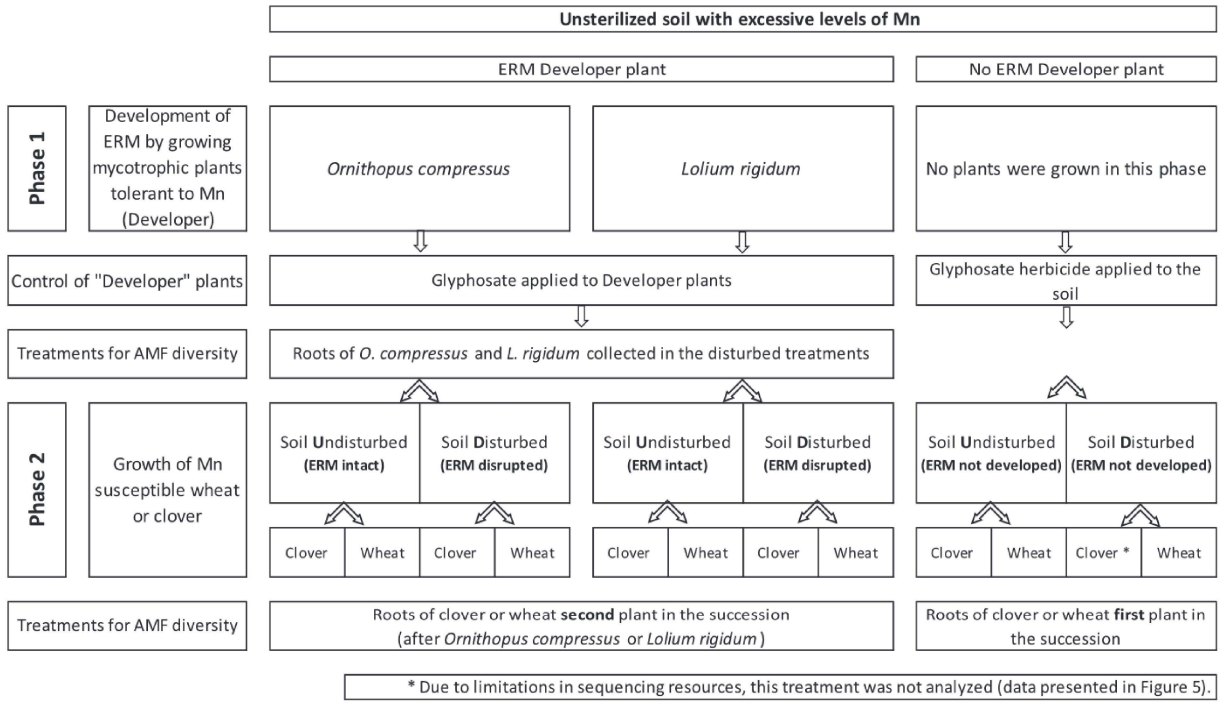

Fig. 1. Combined schematic representation of Experiments 1 (Brito et al., 2014) and 2 (Alho et al., 2015). The root samples used for the AMF diversity studies (Fig. 5) are also identified in this figure. Root samples of *Lolium rigidum* and *Ornithopus compressus* were collected at the end of Phase 1 (identified as *Ornithopus* and *Lolium* in Fig. 5). Treatments where root samples of wheat or subterranean clover (as first or second plant in the succession) are also identified.

presented in Fig. 1.

In Brito et al. (2014) and Alho et al. (2015) protection against excessive levels of Mn was only achieved when Developer plants were *Ornithopus* or *Lolium* and the treatment was Undisturbed soil, that is, an intact ERM was present in the soil at planting of wheat or clover. Whenever the soil was disturbed at the beginning of Phase 2, the protection against Mn toxicity was lost. When a non-mycotrophic Developer was grown in Phase 1, soil disturbance did not affect the results or the types of AMF propagule present in the soil in the beginning of Phase 2. Consequently, in this paper the results were grouped according to the AMF propagule type. As significant differences were observed between mycotrophic Developers in undisturbed soil (ERM intact), these data are presented separately. Therefore, the treatments were grouped as: (A) mainly spores (includes the negative control - no Developer - and non-mycotrophic Developers with and without soil disturbance); (B) spores, colonised root fragments and fragmented mycelium (includes disturbed soil treatments after mycotrophic Developers); (C) intact ERM associated with *L. rigidum*; (D) intact ERM associated with *O. compressus*.

To investigate the influence of plant succession (mycotrophic Developer followed by the test crop) and the type of propagules (determined by the level of soil disturbance) on the AMF diversity present in the roots of wheat and clover, biological samples were taken from Experiment 1 and 2. The root samples of the mycotrophic Developers were obtained from the disturbed soil treatments at the end of Phase 1 and the root samples of wheat and clover were obtained at the end of Phase 2 of Experiment 1 and 2, respectively (see Fig. 1 for treatment codes of wheat and clover). The AMF diversity was assessed by 454 pyrosequencing of the LSU-D2 rDNA genes. The details of technical procedures are fully described in Brigído et al. (2017).

2.2. Protection against biotic stress

In this study, the test crop was tomato (*Lycopersicon esculentum*) and the stressor was *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Experiment 3 was a two-phase pot experiment, similar to those described above, carried out under a controlled environment. The temperature control of the greenhouse only allowed regulation of maximum temperature, which was set at 30 °C. Plants were watered to field capacity daily by weighing the pots. Phase 1 involved the establishment of a Developer plant in unsterilized soil to create ERM formed by indigenous AMF. In this case the Developer plant had not only to be mycotrophic but also could not be susceptible to the disease organism. *L. rigidum* was selected as the ERM Developer and was grown for 8 weeks. At the end of this period it was treated with a systemic herbicide. For half of the pots the soil was then sieved (Disturbed soil treatment) to disrupt the ERM but in the remaining pots the soil was left undisturbed (Undisturbed soil treatment) so that the ERM remained intact. Three pre-germinated tomato seedlings were planted per pot and the developing roots of each plant was inoculated with *F. oxysporum*, using 1 ml per plant of a suspension with a concentration of 0, 10^3, 10^6 or 10^9 conidia ml^{-1}. The inoculation of tomato was performed immediately before the plants were placed in the pots. Nutrients were added to the soil at planting of tomato to ensure that the strategy was compatible with the large application of fertilizer, currently the typical agricultural practice in tomato production. All pots received the same amount of Ca (1000 mg kg^{-1} dry soil as dolomitic limestone with 10% Mg, equivalent to 2600 kg Mg ha^{-1}), N (7.9 mg N kg^{-1} as ammonium nitrate, ~20 kg N ha^{-1}), K

(32 mg K kg^{-1} as potassium sulfate, ~80 kg K ha^{-1}), Zn (3.8 mg Zn kg^{-1} as zinc sulfate, ~10 kg Zn ha^{-1}) and B (3.8 μg kg^{-1} as sodium borate). Tomato was grown for 3 weeks before shoot dry weight, arbuscular colonization (AC) and disease incidence (DI) were evaluated. Disease incidence was assessed visually from no visible symptoms (Score = 1) to stem fully affected (Score = 4).

In an area where *F. oxysporum* was a major constraint to tomato production a field investigation (Experiment 4) was established to test the strategy considered in the pot Experiment 3. The ERM Developer was barley (*Hordeum vulgare*) grown as a cover crop over the winter. Due to soil compaction associated with the harvesting of tomato, a subsoiling operation was necessary to prevent restricted root development. Traditionally this operation is performed in spring immediately before land preparation and bed formation for tomato planting. However, in this experiment, all these operation were performed soon after previous tomato harvesting and before the seeding of the barley to ensure that the ERM associated with barley was keep intact. The cover crop was eliminated by herbicide and only a very superficial tillage was carried out before tomato was planted (see Goss et al., 2017 for details).

3. Results and discussion

3.1. Protection against abiotic stress

Arbuscular colonization (AC) started earlier and developed faster, both in wheat and subterranean clover, when an intact ERM was the preferential source of inoculum (Table 1) and resulted in a significant improvement of plant growth (Table 2). These two variables were significantly correlated (Fig. 2). Soil disturbance *per se* did not affect the results in Experiments 1 and 2 when a non-mycotrophic Developer was grown in Phase 1 (Brito et al., 2014 for wheat and Alho et al., 2015 for subterranean clover). Therefore, the negative effects of soil disturbance observed in both parameters (differences between B and C or D) are due to ERM disruption and not to any changes related to the soil condition. The ability of AMF to protect plants against Mn toxicity was lost when the only propagules present in the soil were spores or spores and colonized root fragments (groups A and B). When an intact ERM was present at the time of planting wheat or subterranean clover, i.e. after *O. compressus* (group D) and *L. rigidum* (group C), there was a significant reduction in the concentration of Mn in the shoots of wheat and the roots of subterranean clover (Table 3). The growth of wheat was significantly negatively correlated with Mn concentration in the shoots (Fig. 3). For subterranean clover the Mn concentration in the shoots was not significantly affected by treatment (Table 3). However, there was a significant effect of the condition of the ERM (present or absent; intact or disrupted) on the Mn concentration in the roots (Table 3), which was significantly and negative related to shoot growth (Fig. 3). For subterranean clover the protection against an excessive level of Mn seems to involve the tripartite symbiosis between AMF, rhizobia and plant. In the presence of an intact ERM the nodule dry weight and the N fixation were enhanced, supporting the view that better growth of the plant depended on improving conditions for the rhizobial bacteroids in the root nodules (Fig. 4). This is consistent with subterranean clover being relatively tolerant to Mn, when it is not depending on biological N fixation.

Table 2
Shoot dry weight of wheat and subterranean clover shoots 21 d after planting. Colonization by indigenous AMF was initiated by different propagules: A – spores only; B – spores, fragmented mycelium and colonized root fragments of *Lolium rigidum* or *Ornithopus compressus* (Disturbed soil); C – spores and intact extraradical mycelium associated with the roots of *L. rigidum* (Undisturbed soil); D - spores and intact extraradical mycelium associated with the roots of *O. compressus* (Undisturbed soil). The soil contained 22.6 mg Mn kg^{-1}. Small letters within the same column separate significant difference ($p < 0.05$). Experiments 1 and 2, results for wheat adapted from Brito et al., 2014 and for subterranean clover from Alho et al., 2015.

	Shoot dry weight (mg $plant^{-1}$)	
	Wheat	Subterranean clover
A	96.3 c	34.1 c
B	88.3 c	34.8 c
C	157.6 b	64.4 b
D	252.8 a	104.5 a

The earlier and faster AMF colonization when initiated by an intact ERM is well established in the literature (Goss et al., 2017). However, the benefits for the host plant, in terms of P acquisition and shoot growth are only present in the early stage of plant growth and tend to disappear with time because reserves in the soil are not limiting growth (Miller, 2000). The role of AMF in protecting the host plant against abiotic stresses is also well documented (Goss et al., 2017), but the degree of protection achieved depends on the level of colonization of the host plant at the time it faces the stress. The results shown above indicate that under marginal soil conditions, with a small P content and excessive levels of Mn, the role of AMF in protecting the host plant depends on early colonization associated with the presence of an intact ERM as the preferential source of AMF propagule. Considering that there are many abiotic stresses when seeds germinate, the presence of an intact ERM in the soil might play a decisive role in agricultural ecosystems. Moreover, the natural P content of most soils is small, reserves of P in the world are decreasing, and farmers from many parts of the world are unable to purchase fertilizers at current prices.

It is important to recognise that the shoot growth of wheat and subterranean clover was significantly enhanced after *O. compressus* compared with that after *L. rigidum* (Table 2), despite the fact that AC (Table 1) and Mn concentrations were similar after both

Table 1
Arbuscular colonization (AC) of wheat by indigenous AMF 10 (W 10 DAP) and 21 (W 21 DAP) days after planting (DAP), and subterranean clover 21 (C 21 DAP) DAP. Colonization was initiated by different propagules: A – spores only; B – spores, fragmented mycelium and colonized root fragments of *Lolium rigidum* or *Ornithopus compressus* (Disturbed soil); C – spores and intact extraradical mycelium associated with the roots of *L. rigidum* (Undisturbed soil); D - spores and intact extraradical mycelium associated with the roots of *O. compressus* (Undisturbed soil). The soil contained 22.6 mg Mn kg^{-1}. Small letters within the same column separate significant difference ($p < 0.05$). Experiments 1 and 2, results for wheat adapted from Brito et al., 2014 and for subterranean clover from Alho et al., 2015.

	Arbuscular colonization (AC) (% root length colonized)		
	Wheat		Subterranean clover
	10 DAP	21 DAP	21 DAP
A	0.47 b	16.6 b	26.2 b
B	0.56 b	15.8 b	32.0 b
C	5.34 a	49.8 a	61.4 a
D	8.52 a	54.8 a	68.4 a

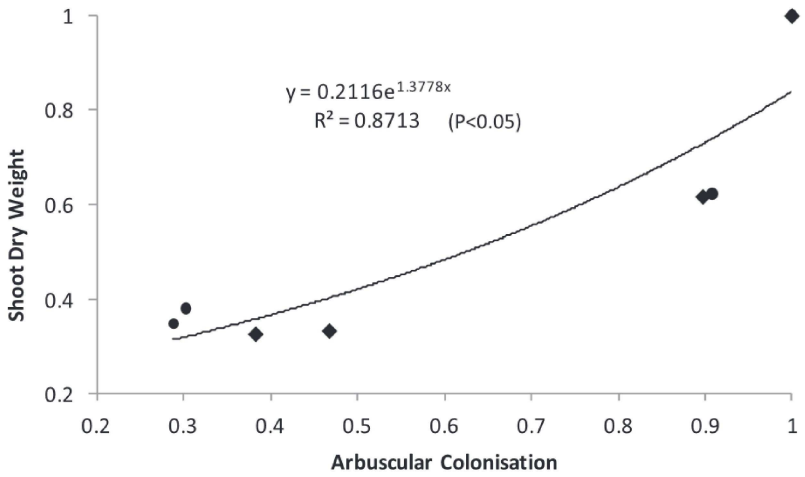

Fig. 2. Relationship between arbuscular colonization and shoot dry weight at 21 d after planting for wheat (circles) (Experiment 1; Brito et al., 2014) and subterranean clover (squares) (Experiment 2, Alho et al., 2015). Values for both variables are expressed as a proportion of the maximum achieved by each crop.

Table 3
Effect of the native AMF propagule type in the soil at the time of planting on the manganese concentration in wheat shoots (Ws Mn) and in subterranean clover shoots (Cs Mn) and roots (Cr Mn) at 21 d after planting. Colonization by indigenous AMF was initiated by different propagules: A – spores only; B – spores, fragmented mycelium and colonized root fragments of *Lolium rigidum* or *Ornithopus compressus* (Disturbed soil); C – spores and intact extra radical mycelium associated with the roots of *L. rigidum* (Undisturbed soil); D - spores and intact extra radical mycelium associated with the roots of *O. compressus* (Undisturbed soil). The soil contained 22.6 mg Mn kg^{-1}. Small letters within the same column separate significant difference ($p < 0.05$). Experiments 1 and 2, results for wheat adapted from Brito et al. (2014) and for subterranean clover from Alho et al. (2015).

	Manganese concentration (mg kg^{-1})		
	Ws Mn	Cs Mn	Cr Mn
A	181 a	141.2	239.7 a
B	185.8 a	134.7	225.9 a
C	112.5 b	124.7	133.5 b
D	107 b	129.0	107.3 b

Developer plants (Table 3). This is consistent with the effect of the Developer plant and the condition of ERM on the AMF diversity found in the roots of wheat and clover (Fig. 5). When an intact ERM was not a propagule source, the structure of the AMF community in roots of the Fabaceae (*O. compressus* and *Trifolium subterraneum*) and Poaceae (*L. rigidum* and *Triticum aestivum*) were more similar within each plant family but significantly different between plant groups. It is widely recognized that preferential associations between AMF and host plants exist, at least at the family level (Johnson et al., 1991, 1992; Scheublin et al., 2004; Bever et al., 2009). However, the presence of an intact ERM associated with the first plant, acting as the preferential source of AMF propagule, shifts the structure of AMF community present in the second plant (*T. subterraneum* or *T. aestivum*) to correspond with that of the first plant of the succession independently of their botanical group (Fig. 5 - higher similarity between AMF communities from *O. compressus* and wheat grown after *O. compressus* in undisturbed

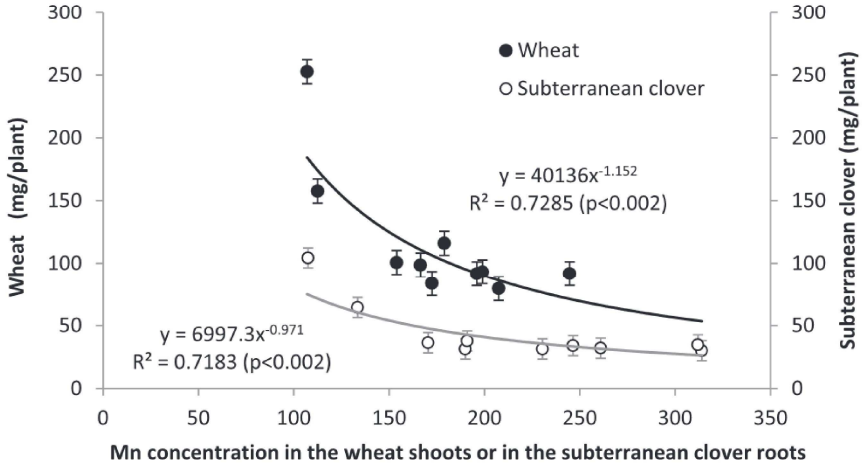

Fig. 3. Relationship between Mn concentration in the shoots of wheat or roots of subterranean clover (ppm) and the respective shoot dry weight, 21 d after planting. The soil contained 22.6 mg Mn kg-1. Experiments 1 and 2, results for wheat adapted from Brito et al., 2014 and for subterranean clover from Alho et al., 2015.

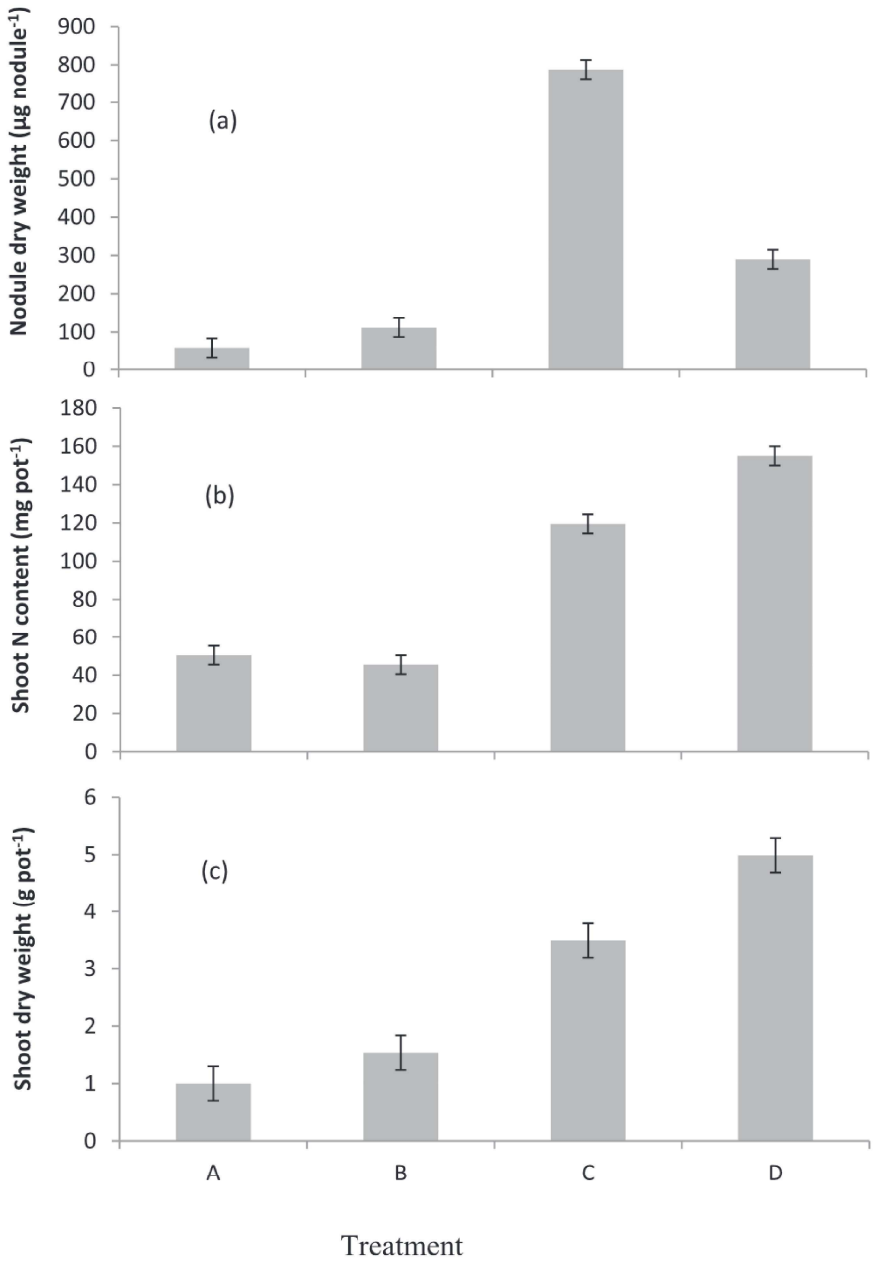

Fig. 4. Effect of the native arbuscular mycorrhiza propagules type in the soil at the time of planting on (a) Nodule dry weight, (b) shoot N content and (c) shoot dry weight of subterranean clover at 6 weeks after planting. Treatment A – spores only; B – spores, fragmented mycelium and colonized root fragments of *Lolium rigidum* or *Ornithopus compressus* (Disturbed soil); C – spores and intact extraradical mycelium associated with the roots of *L. rigidum* (Undisturbed soil); D - spores and intact extraradical mycelium associated with the roots of *O. compressus* (Undisturbed soil). The soil contained 22.6 mg Mn kg-1. Experiment 2, results adapted from Alho et al., 2015.

soil treatment or *L. rigidum* and subterranean clover after *L. rigidum* in undisturbed soil treatment).

Functional diversity associated with AMF is identified in several situations regarding abiotic stresses, including protection against excessive levels of metalloid ions (Clark et al., 1999; Yang et al., 2015). In the present study, AMF colonization rate and Mn concentration in the shoots of the wheat or the roots of subterranean clover were similar, when the plants were preferentially colonised by an intact ERM previously developed in the soil with *O. compressus* or *L. rigidum*. But the growth of the follow-up plant was significantly greater when *O. compressus* was the Developer plant. The two Developer plants presented AMF communities with dissimilar structures that predominantly colonised the roots of the second host plant when the ERM was kept intact. Therefore, it is reasonable to infer that the better growth of wheat and clover after *O. compressus* was associated with more beneficial functional traits of the AMF assemblage with this specific Developer, in the presence of an intact ERM.

The biological diversity among AMF is far from being completely known but is certainly huge, and there is plenty of evidence of functional diversity among them. There is even evidence that the benefits depend on the AMF assemblage present in the roots of the host plant (Barea et al., 2011). Host plant preferences handicap the practical exploitation of the use of AMF to protect cultivated plants against abiotic stresses. Our findings indicate that it is possible to manage the AMF biological diversity present in the roots of a host

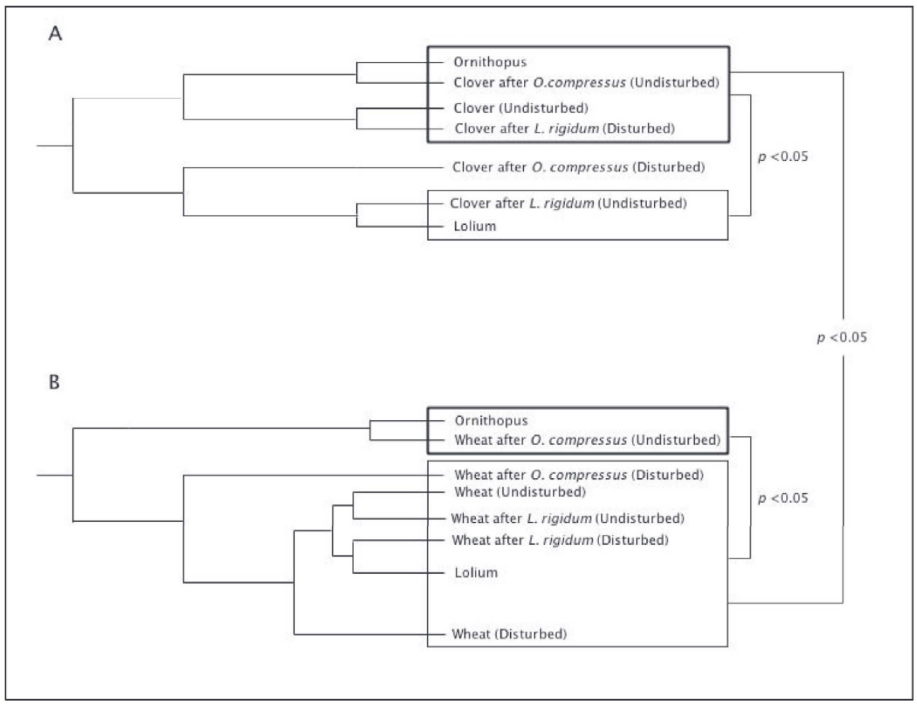

Fig. 5. Dendrogram representing the similarity of AMF communities in the root samples based on the structured-based Bray-Curtis dissimilarity coefficient. (A) Root samples from *Lolium* (*Lolium rigidum*); *Ornithopus* (*Ornithopus compressus*); Clover as first plant in the succession grown in undisturbed soil (Clover Undisturbed); Clover as second plant grown after *L. rigidum* in disturbed and in undisturbed soil; Clover as second plant grown after *O. compressus* in disturbed and in undisturbed soil; (B) Root samples from Wheat as first plant in the succession grown in undisturbed and in disturbed soil; Wheat as second plant grown after *L. rigidum* in disturbed and in undisturbed soil; Wheat as second plant grown after *O. compressus* in disturbed or in undisturbed soil; *Lolium* (*Lolium rigidum*); *Ornithopus* (*Ornithopus compressus*).
The significant differences detected with permutational multivariate analysis of variance between the AMF community structures are indicated in the figure. No significant differences were detected between the AMF community patterns within each rectangle. Experiment 1 and 2, results from Brigído et al. (2017).

plant by the choice of the previous plant in a succession and the maintenance of the ERM integrity, opening the possibility for the management of AMF functional diversity to protect cultivated plants against abiotic stresses.

3.2. Protection against biotic stress

In Experiment 3, the investigation of a biotic stress in a pot experiment, inoculation with *F. oxysporum* significantly reduced AC of tomato, thereby indicating competition between the two fungi (Fig. 6), as recognized by Wehner et al. (2010). However, the ability of AMF to compete with *F. oxysporum* was greater when an intact ERM was the preferential inoculum source, which is consistent with an earlier and faster colonization granted by this type of inoculum (Goss and de Varennes, 2002; Brito et al., 2013).

This timely colonization was crucial in the reduction of disease

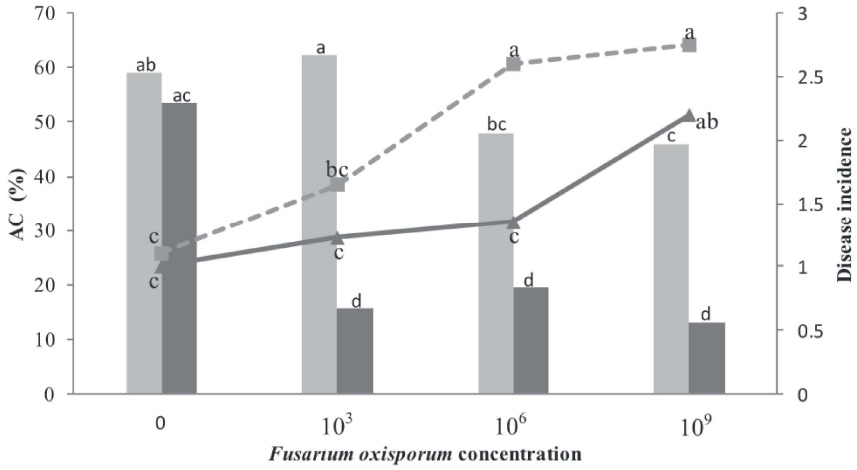

Fig. 6. Effect of the presence of an intact (light grey bars) or disrupted (dark grey bars) ERM of AMF at the time of tomato planting on the arbuscular colonization (% AC, left axis) and disease incidence (right axis - dotted line for disrupted ERM and solid line for intact ERM), 16 d after planting. Plants inoculated with conidia of *Fusarium oxysporum*: 0, 10^3, 10^6 or 10^9 conidia per plant. For each parameter values with the same letter are not significantly different from each other ($p = 0.05$). Experiment 3.

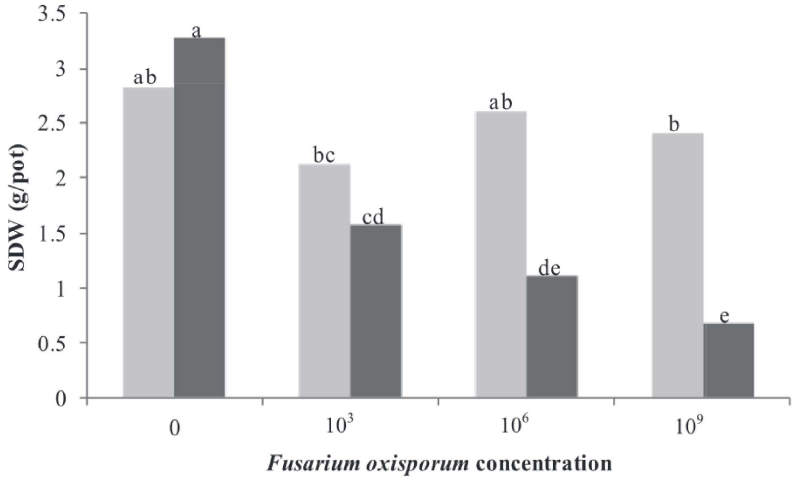

Fig. 7. Effect of the presence of an intact (light grey) or disrupted (dark grey) ERM of AMF at the time of tomato planting on the shoot dry weight, 16 d after planting. Plants inoculated with conidia of *Fusarium oxysporum*: 0, 10^3, 10^6 or 10^9 conidia per plant. The same letter indicates that values are not significantly different from each other ($p = 0.05$). Experiment 3.

incidence (Fig. 6) and increase of plant growth (Fig. 7). When an intact ERM was present in the soil at tomato planting, growth was not significantly affected by inoculation with 10^3 to 10^9 conidia and was greater at the highest concentration of *F. oxysporum* inoculation (10^9), than the one observed in the disturbed soil treatment (ERM disrupted) at the lowest inoculation rate (10^3). The protection to the initial tomato growth observed in the pot experiment was kept until the end of the plant cycle in the field experiment and significantly improved the final tomato yield (Fig. 8). As the colonization rate of tomato plants in the field experiment was not assessed, the mechanisms associated with the protection observed can only be inferred from the results of the pot experiment. However, it is unlike that the benefits of the cover crop could result from any change in the nutrient availability, considering the level of fertilizer and other inputs (irrigation, pesticides) applied to the crop. All the production techniques used in the field experiment (irrigation, fertilizer application, pesticides for crop protection) were of the standard used for achieving high yields. The only adaptation of the production techniques was related to the need to develop the ERM and the maintenance of its integrity. The ERM was developed by a cover crop (barley), and kept intact by a minimum soil disturbance for planting the tomato. Therefore, primary deep tillage and the formation of tomato seedbeds were performed in autumn before the seeding of the cover crop. The cover crop was eliminated by herbicide and only superficial tillage was necessary to insert the plugs containing the tomato seedlings.

Soil borne pathogens are a major limitation for intensive crop production and cause losses as great as 50% (Lewis and Papavizas,

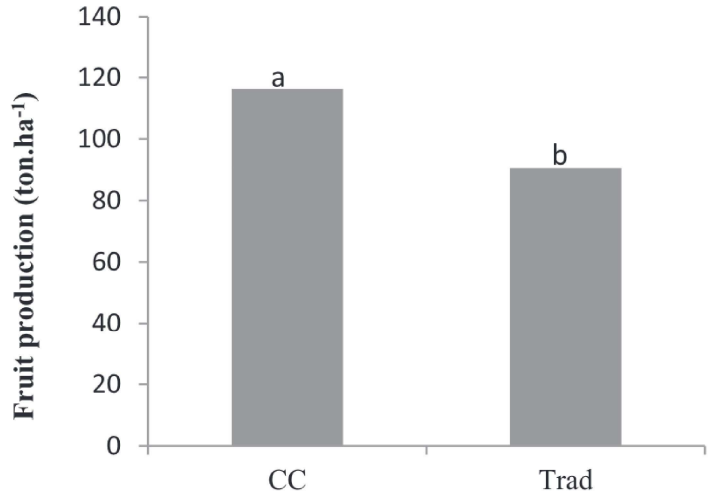

Fig. 8. Total yield of tomato in a field with *F. oxysporum* present. CC - cover crop (barley) and Trad. - traditional land preparation with no cover crop. Different letters indicate values differ significantly ($p \leq 0.05$). Experiment 4.

1991). Moreover, the environmental concerns associated with the use of pesticides are limiting other options for their control under field conditions. The use of AMF to protect the crops has only been successful when AMF are inoculated in advance of the pathogen infection (Torres-Barragan et al., 1996; Sikora et al., 2008). Previous inoculation with AMF is only possible at the nursery stage for transplanted crops. Our findings show that there is an alternative strategy for crops that are seeded directly in the field.

4. Concluding remarks

In combination, the results reported here suggest that the constraints to the intentional use of AMF in the cropping systems can be overcome. The use of indigenous AMF communities solves the problems associated with the cost and low biodiversity of commercial inoculum. The use of appropriate tillage techniques allows the maintenance of intact ERM as the preferential AMF propagule and permits earlier and faster colonization, which is crucial when protection, either to biotic or abiotic stresses is required. The choice of a host plant to develop the ERM can be used as a tool to select and take advantage of more beneficial AMF consortia from the available functional diversity, because, in the presence of an intact ERM, preferential associations of the plant host and AMF are changed. The results of the field experiment with tomato indicates that the proposed strategy can be effective, even with the current level of inputs needed to achieve high yields, which is often considered a limitation for full exploitation of the benefits of the symbiosis.

Soil acidity and related toxicity of metalloid ions like Mn^{2+} or Al^{3+} are major constraints on a global scale, with limited P availability also being frequently associated. Owing to economical limitations, farmers from large regions of the world, where an increase in food production is more urgent, do not have access to inputs of P. The role of AMF in the protection of crop plants against these stresses can be of great significance in closing yield gaps in these regions and should be considered in a strategy within the sustainable intensification of agriculture. In developed countries the problems of sustainable intensification are more related to the need of reducing the environmental impact of agricultural systems. Soil borne diseases in intensive production systems have a tremendous economic and environmental impact due to yield reduction and the use of pesticides. The strategy proposed in this paper, relying on the indigenous AMF population, proved to be efficient in solving this problem and at the same time allowing high yields.

There are several opportunities within the cropping systems to develop the ERM of the indigenous AMF population in the soil. Weeds, elements of the crop rotation and cover crops, if mycotrophic, can all act as host plants and play this role. Appropriate tillage techniques (no-tillage and reduced tillage) must be implemented to keep ERM intact at the seeding of the crop to be protected. When deep soil disturbance is needed to solve problems, such as soil compaction, primary tillage operations need to be anticipated and carried out before seeding the Developer plant.

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