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The effect of plant mycotrophy and soil disturbance on soil microbial activity

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Abstract

In cropping systems, the choices adopted for the tillage system used and plants cultivated can strongly influence the soil microbial population and its functional profile. Arbuscular mycorrhizal fungi are an important component of soil microbiome and their mutualistic symbiosis with the majority of higher plants grant the latter a wide range of benefits. The extraradical mycelium developed by these fungi expands the volume of soil influenced and harbours a diversity of microbes establishing a distinct environment of complementary interactions. We assessed how growing plants with different levels of mycotrophy modifies the biological activity profile in the soil under Mn toxicity and whether this is modified by soil disturbance. Following mycotrophic plants, soil contained a more active microbiome than after the non-mycotrophic plants, as expressed by higher values of soil basal respiration or dehydrogenase activity. Additionally, the count of phosphorus solubilizes and activity of phosphatase were greater after mycotrophic plants. Even among mycotrophic plants, different profiles of biological activity can be distinguished after growing a legume or grass. ERM disruption by soil disturbance decreased most of the parameters studied and for phosphatase activity and P solubilizers in a more significant way. These results indicate that even under Mn toxicity, the microbiome associated with AMF symbiosis following mycotrophic plants growth presented a higher biological activity and had a differential biological response towards the stress imposed by soil disturbance, when compared with the microbiome associated with non-mycotrophic roots.

KEYWORDS

arbuscular mycorrhizal fungi, functional diversity, soil enzymes, tillage

1 | INTRODUCTION

Soil microbiomes are essential to the maintenance of biogeochemical processes and play a decisive role in the cycling of nutrients and therefore in their availability for plant growth (Marschner, 2002). Microbiome responses to different types of disturbance and stress could act as functional indicators of agricultural management practices (Zilli et al., 2003). Functional diversity includes the multiplicity of microbial activities in soils and is closely linked to ecosystem stability (Hampp & Tarkka, 2009). Among soil microorganisms, the most ubiquitous are mycorrhizal fungi that form an ancient and widespread symbiosis with vascular plants. Within that group, the most important for agricultural crops are the arbuscular mycorrhizal fungi (AMF) (Goss et al., 2017). The symbiosis between AMF and higher plants also includes a network of extraradical mycelium (ERM) that extends beyond the rhizosphere. This network allows the exploitation of a larger amount of soil and enhances the acquisition of nutrients, including nitrogen and phosphorus, especially those that have limited mobility (Duponnois et al., 2008; Goss et al., 2017). The zone influenced by mycorrhizal roots and the extraradical hyphae is called the mycorrhizosphere (Linderman, 2008). Other benefits accrued by the host plant include tolerance to biotic and abiotic stress (Begum et al., 2019; Brito et al., 2014, 2019), modification of plant gene expression (Balestrini & Lanfranco, 2006), modulation of the host defence system (García-Garrido & Ocampo, 2002) [8] and the hormonal balance to improve plant growth (Chanclud & Morel, 2016) [9]. AMF also increase soil stability through the formation of microaggregates important for soil structure and necessary for optimum root development (Barea et al., 2011).

Biological activity in the rhizosphere is modified when AMF are present, as these fungi reduce root exudation as well change it qualitatively. In consequence, the surrounding microbial communities can also be affected (Andrade et al., 1997) such that the microbial activity associated with the rhizosphere of mycorrhizal and non-mycorrhizal plants could be completely different (Akyol et al., 2019; Garbaye, 1991; Offre et al., 2007). Studies comparing the rhizosphere microbiome between mycotrophic and non-mycotrophic plants show differences in functional groups of microorganisms, such as P solubilizers (Timonen & Marschner, 2006) and Mn-oxidizers (Nogueira et al., 2007). Thus, indicating that the ERM formed in AMF symbiosis acts as an important environment for microbial survival.

Soil microorganisms are also greatly influenced by various abiotic factors, such as pH, soil moisture, oxygen availability and soil texture. These parameters are likely to change with tillage regime, including crop residue management, and therefore influence soil microbial communities (Degrune et al., 2017). Tillage can affect the amount and distribution of soil organic matter (SOM) and alter the physical and chemical properties of soil environment by affecting water content and aeration. Conventional practices with intensive tillage decrease the diversity of community structure, disrupt nutrient cycling and result in less stability or resilience of soil functional status (Smith & Collins, 2007). By changing SOM content, cropping practices could also shift the balance of rhizosphere and mycorrhizosphere communities in biodiversity and function.

Some quantitative assessments have been generally accepted for evaluating changes in soil functional activity. For example, soil microbial biomass, carbon and nitrogen content and soil respiration have been widely used as indicators of soil biological status (Vogel et al., 2019). Metabolic quotient represents the metabolic status of soil microorganisms, in which larger values indicate greater stress conditions, but it has to be interpreted with caution because an increase could also indicate an input of easily degradable carbon that stimulates microbial activity (Cardoso et al., 2013).

The activity of soil enzymes is considered indicative of specific biochemical reactions of the entire soil microbial community involved in SOM mineralization (Klose & Tabatabai, 1999). In consequence, microbial response in experiments has commonly been evaluated by soil enzyme activity (Xiao et al., 2018). The most studied soil enzymes belong to the classes of oxidoreductases and hydrolases (Dotaniya et al., 2019). Dehydrogenase (EC 1.1.1.) is a group of oxidoreductase isoenzymes used as an indicator of general soil microbial activity because it occurs intracellularly in all living microbial cells. It plays a significant role in the biological oxidation of SOM and is assumed to be proportional to the biomass of soil microorganisms (Wolinska et al., 2012). Arylsulfatase (arylsulfate sulfohydrolase, EC. 3.1.6.1), β-glucosidase (1,4 − D- glucosidase, EC 3.2.1.21) and phosphatase (phosphoric monoester hydrolases, EC 3.1.3) are key hydrolase enzymes involved in SOM mineralization (sulfur, carbon and phosphorus, respectively) by hydrolyzation of organic compounds into inorganic forms (Deng & Tabatabai, 1996; Klose et al., 1999). Therefore, the activities of these soil enzymes could be used as an indicator of functional profile and microbial status of soil management (Dick & Burns, 2011; Gianfreda & Ruggiero, 2006). However, owing to its substrate specificity, the enzymatic activity should not be assessed as an individual parameter to determine microbiological activity indices (Alkorta et al., 2003). Microbial activity in soils is crucial to soil function and studies that link diversity and function in different ecosystems are important in predicting the outcome of specific soil management interventions.

Brito et al. (2014) proposed a strategy for managing AMF based on selecting host plants (Developer plants) for the intentional development of an extensive ERM, which, when kept intact by the adoption of appropriate tillage techniques, acts as the preferential source of inoculum for the following crop. Colonization from ERM occurs earlier and faster than from spores, so protecting the new crop against biotic and abiotic stresses existing in the soil. An understanding of how microbial communities respond to different agricultural practices and perturbations is important to maximize the sustainability of soil resources (Bissett et al., 2013). Therefore, the present work aims to understand the effect of the strategy proposed by Brito et al. (2014) on the growth of the ERM Developer plant and on the functional activities of the remaining soil microbiota. We assessed the effect of plant type, according to their level of mycotrophy and soil disturbance, on the profile of (i) microbial functional groups, (ii) enzymatic activity and (iii) soil microbiological attributes.

2 | MATERIALS AND METHODS

2.1 | Experimental design

A pot experiment was performed in a greenhouse under controlled conditions from January to April 2019. We used a sandy, acidic soil (sandy loam Eutric Cambisol – FAO) collected from the top 20 cm of a natural pasture at Herdade da Mitra-University of Évora, Alentejo, Portugal (38° 32´ N; 08° 00´ W), having an organic C content of 10.5 g.kg^{-1}, a pH of 4.8 in water, the ammonium acetate exchangeable manganese content at pH 7 was 29±4 μg.g^{-1}, and previously described by Goss and Carvalho (1992) as causing Mn toxicity in wheat. This soil is characterized by a high AMF diversity (Alho et al., 2015; Brígido et al., 2017; Brito et al., 2014). It was homogenized by sieving to ensure that all treatments had the same initial conditions and then packed into 8 kg pots. Three common arable plant species, widespread in the Mediterranean basin, were sown in 8 replicate pots, with 5 plants per pot. Two species, *Ornithopus compressus* L. (a legume) and *Lolium rigidum* Gaudin (a grass) known to be mycotrophic and the non-mycotrophic *Silene gallica* L. To avoid confounding effects, weeds were controlled by hand on a daily basis, and all the pots were watered approximately to field capacity (0.17 g.g^{-1}) by weight. The plants grew for 11 weeks, after which their aerial parts were severed from the roots in all pots. For the disturbed treatment, the soil in half of the pots of each species was subjected to mechanical disturbance by passing through a 4 mm sieve to disrupt the extra radicular mycelium. Root material was collected during

this process and their colonization by AMF determined after staining with trypan blue, according to the magnified intersections method (McGonigle et al., 1990). The soil and unused roots were mixed, repacked into the same pots and shoot material was returned to the soil surface. The remainder of the pots of each species formed the undisturbed treatment and shoot material was also returned to the soil surface. All pots were then left for 10 days. Soil was sampled to assess biological activity at three phases of the experiment: the first before planting (bulk soil), the second, 11 weeks after plant growth to see the effect of plant type and the third sampling 10 days after soil disturbance to see the effects of soil disturbance. Sampled soil was passed through a 2 mm sieve and the functional activity was measured in terms of soil microbial activity, microbial functional counting and enzymatic activity related to organic matter cycling.

2.2 | Soil microbial activity

Water holding capacity and water content were determined (Monteiro & Frighetto, 2000) and the information used in calculating the evaluated parameters. Soil basal respiration (SBR) was measured in a closed jar incubated for 7 days at 26°C (Silva et al., 2007). The CO$_2$ released was adsorbed in NaOH and determined by HCl titration. The results are reported as milligrams of CO$_2$ released per kilogram of soil per hour (Equation 1).

$$SBR = \frac{(Vb - Vs).\,M.\,6.1000}{ds.\,t} \qquad (1)$$

where: Vb was the volume of HCl consumed in the blank (ml); Vs was the volume of HCl consumed in the test sample (ml); M was the HCl molarity; 6 equivalent factor (1 ml of 0.5 N HCl is equivalent to 6 mg C-CO2 in the NaOH solution); ds was the weight of dry soil; t was the time of incubation.

Determination of total microbial biomass carbon (MBC) followed the protocol of fumigation extraction suggested by Vance et al. (1987) in which the soil is fumigated with chloroform in a desiccator and the carbon content calculated following an oxidation reaction with potassium permanganate. The values of MBC are given by the carbon content of fumigated soil minus that of the nonfumigated soils, all divided by the proportion of microbial C evolved (*kc*). A value of 0.45 was used for *kc* in MBC calculation (Equation 2), as recommended by Joergensen (1996).

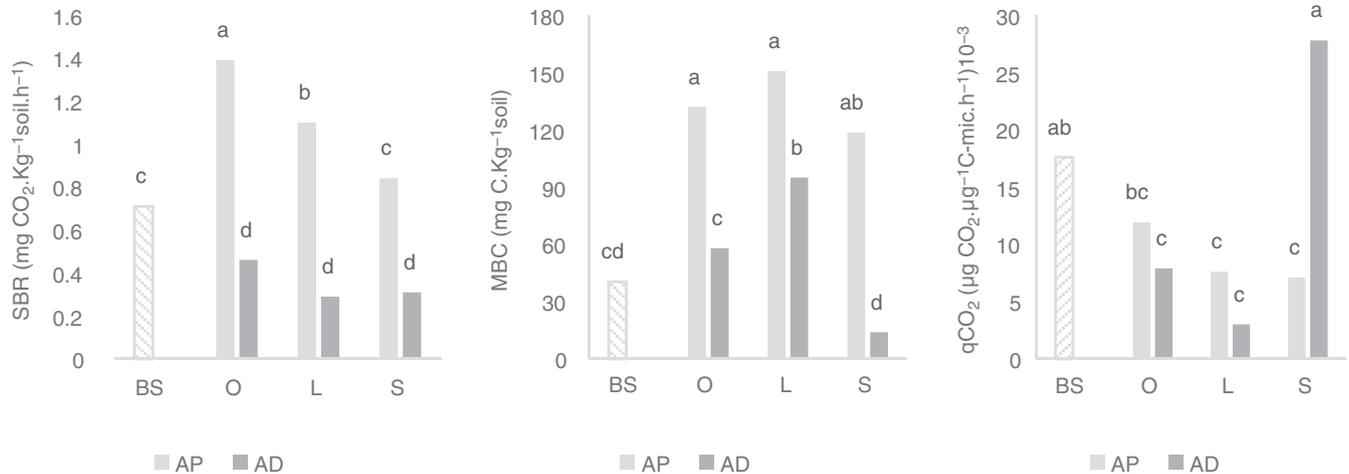$$Cmic = \frac{(Cf - Cnf)}{kc} \qquad (2)$$

FIGURE 1 Effect of the plant species and soil disturbance on soil basal respiration (SBR), microbial biomass carbon (MBC) and metabolic quotient (qCO$_2$). AP: After plant growth sampling; AD: After soil disturbance sampling; BS: Bulk soil; O: *O. compressus*; L: *L. rigidum*; S: *S. gallica*. Means that share different letters indicate significant differences between treatments at the 5% level (Tukey's test).

where: Cf was mg of C per kilogram of fumigated soil; Cnf was mg of C per kilogram of non-fumigated soil; kc was proportion of microbial C evolved (0.45).

The metabolic quotient (qCO$_2$), the ratio between SBR and carbon microbial biomass (Anderson & Domsch, 1990), was used to estimate the efficiency of substrate consumption by microorganisms as a stress indicator when the microbial biomass is affected.

2.3 | Functional groups of culturable microorganisms

Six functional culturable groups of soil microorganisms were evaluated: total bacteria, fungi, ammonifiers, sulfur (S) oxidizers, manganese (Mn) oxidizers and phosphorus (P) solubilizers. For bacteria, fungi, ammonifiers and P solubilizers, the protocols used were the ones described in Albino and Andrade (2007). Mn oxidizers microorganisms were counted according to Nogueira et al. (2007) in Garretesen's media. S oxidizers were counted in thiosulfate broth as suggested by Vidyalakshmi and Sridar (2007) using bromothymol blue as an indicator of pH reduction instead of bromocresol purple. Ammonifiers and sulfur oxidizers are presented as the logarithm of most probable number per gram of soil (logMPN.g^{-1}) and the others as the logarithm of colony forming units per gram of soil (logCFU.g^{-1}).

2.4 | Enzymatic activity

Dehydrogenase was measured according to Casida et al. (1964) with modifications. Soil (5 g) was incubated with 1% 2,3,5-triphenyltetrazolium chloride (TTC) (5 ml) for 24 h at 37°C. Triphenyl formazan (TPF) formed by the reduction

of TTC under dehydrogenase activity during incubation was extracted from the soil with 20 ml of methanol and left to decant for about 10 min. The supernatant was centrifuged at 5000 rpm for 5 min and then 3 ml were transferred to cuvettes and determined by spectrophotometry ($\lambda = 485$ nm) in triplicate (Frighetto, 2000). The arylsulfatase, β-glucosidase and phosphatase activity were measured according to ISO 20130:2018 (ISO, 2018) in 96-well microplates. After the incubation time appropriate to each enzyme (240 min for arylsulphatase, 120 min for β-glucosidase and 30 min for phosphatase), their respective substrates (potassium ρ-nitrophenyl-sulphate, ρ-nitrophenyl-β-D-glucopyranoside and ρ-nitrophenyl-phosphate) were hydrolyzed into a yellow coloured ρ-nitrophenol and all determined by spectrophotometry ($\lambda = 405$ nm).

2.5 | Statistical analysis

The experimental design was a complete randomized block with four replicates. The treatments were in factorial combination and consisted of two factors: plant type (with 3 levels) and soil disturbance (with 2 levels). ANOVA was performed based on the two factors using a generalized linear model, and Tukey's test at 5% level was used to compare the means using the software Minitab 21® (Minitab., 2021).

3 | RESULTS

3.1 | Root colonization rate by AMF

To confirm the mycotrophic level of the plants used in this study, the root colonization by arbuscular mycorrhizal fungi (AMF) was assessed. *O. compressus* and

TABLE 1 Effect of the plant species and soil disturbance on soil microbial functional group counts of fungi, ammonifiers and sulphur oxidizers.

	Fungi (log CFU.g⁻¹)				Ammonifiers (log MPN.g⁻¹)				Sulfur oxidizers			
	BP	AP	AD	Mean plant	BP	AP	AD	Mean plant	BP	AP	AD	Mean plant
Plants												
O. compressus	4.91	5.02	5.12	5.02	4.49	5.39	7.09	5.66	3.06	2.97	3.33	2.83
L. rigidum		5.17	5.15	5.08		5.20	6.68	5.46		2.94	3.33	3.11
S. gallica		5.14	5.03	5.03		4.89	6.13	5.17		2.91	2.11	2.69
Mean soil	4.91 B	5.11 A	5.10 A	A	4.49 C	5.16 C	6.63 B	A	3.06	3.00	2.82	

Note: Means that share different letters indicate significant differences between treatments at the 5% level (Tukey's test).

Abbreviations: AD, after soil disturbance; AP, after plant growth; BP, before planting; CFU, colony-forming units; MPN, most probable number.

L. rigidum showed AMF colonization of 84% ($\pm 0.2\%$) and 75% ($\pm 0.4\%$), respectively. The root colonization by AMF of the former was significantly higher than the latter. No root colonization by AMF was observed in *S. gallica* roots.

3.2 | Soil microbial activity

The mycotrophy of the plant and the soil disturbance strongly affected the soil general microbiological activity (Figure 1). The presence of the any of the plants promoted a great increase in SBR and MBC, relative to the bulk soil. Greater SBR was observed under the mycotrophic plants, but the difference for MBC was not statistically significant ($p > 0.05$). Among the mycotrophic plants, the largest mean value of SBR was observed following growth of *O. compressus* plants and the largest mean value of MBC was found following growth of *L. rigidum*. The results for the metabolic quotient (qCO_2) indicated that among the different plants, smaller values were observed after mycotrophic plants but soil disturbance had no significant effect on the metabolic status under these plants.

Soil disturbance significantly decreased SBR and MBC, irrespective of plant mycotrophy; however, the reduction of SBR was much more evident for mycotrophic than non-mycotrophic plants, whereas the opposite effect was found for MBC. Soil disturbance only had a significant effect on qCO_2 following the non-mycotrophic plant (*S. gallica*).

3.3 | Functional groups of culturable microorganisms

All microbial counts were increased by the presence of the plants relative to values for the bulk soil, with the exception of sulfur oxidizers. Soil disturbance did not affect the mean counts of fungi but increased those for the group of ammonifiers. However, differences were not related to plant mycotrophy (Table 1).

The interaction between plant type and soil disturbance was significant for the groups of total bacteria, P solubilizers and Mn oxidizers (Figure 2). Soil disturbance caused a significant increase in counts of total bacteria and Mn oxidizers in soil following *O. compressus*. Conversely, counts of P solubilizers significantly decreased after growth of the mycotrophic plants in disturbed soil. A distinct pattern was evident in the comparison of soils from under the non-mycotrophic plant, where soil disturbance had no significant effect on the counts of total bacteria, Mn oxidizers or P solubilizers, with those from under mycotrophic plants, where significant negative (reduction in
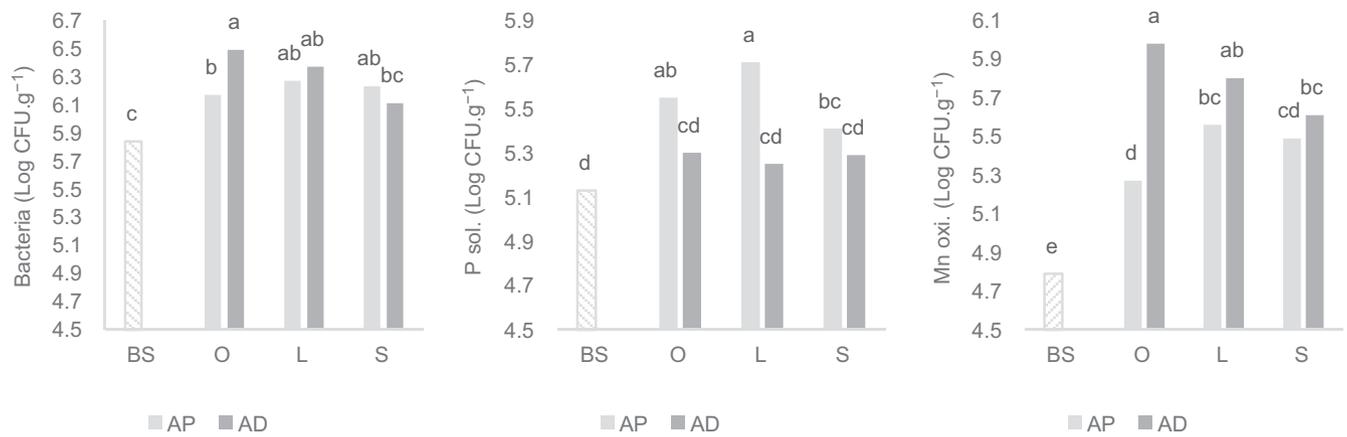
FIGURE 2 Effect of the plant species and soil disturbance on total bacteria, P solubilizers (P sol.) and Mn oxidizers (Mn oxi.). AP: After plant growth sampling; AD: After soil disturbance sampling; BS: Bulk soil; O: *O. compressus*; L: *L. rigidum*; S: *S. gallica*. Means that share different letters indicate significant differences between treatments at the 5% level (Tukey's test).
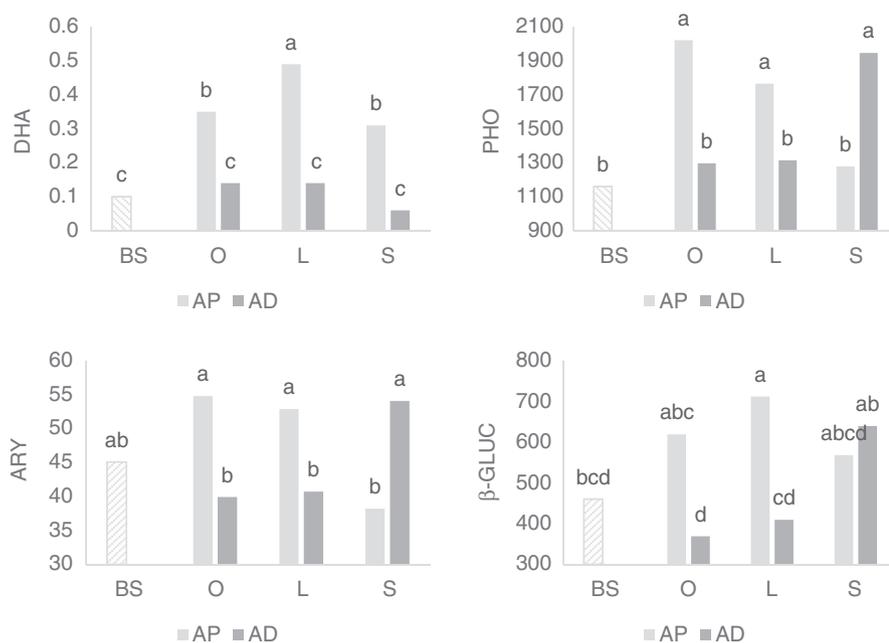

FIGURE 3 Effect of the plant species and soil disturbance on the activity of dehydrogenase (DHA), phosphatase (PHO), arylsulfatase (ARY) and β-glucosidase (β-GLUC); AP: After plant growth sampling; AD: After soil disturbance sampling; BS: Bulk soil; O: *O. compressus*; L: *L. rigidum*; S: *S. gallica*. Values of DHA in μgTPF.g^{-1} dry soil.h^{-1} and values of ARY, PHO and β-GLUC in nmolρ-nitrophenol.g^{-1} dry soil.h^{-1}. Means that share different letters indicate significant differences between treatments at the 5% level (Tukey's test).

P solubilizers) and positive (increases of total bacteria and Mn oxidizers) effects of soil disturbance were found.

3.4 | Enzymatic activity

Enzymatic activity increased between the original bulk soil sampling and the sampling after plant growth (Figure 3). In general, soil disturbance led to a decrease in the mean of all enzymatic activity measured in the study, except for arylsulfatase. The effect of soil disturbance after each plant showed clear differences between mycotrophic and non-mycotrophic plants. Within mycotrophic plants, soil disturbance caused a significant decrease of arylsulfatase, β-glucosidase and phosphatase activity. In contrast, after the non-mycotrophic plant soil disturbance led to an increase in arylsulfatase and phosphatase activity but did

not affect the β-glucosidase activity. The greatest value of dehydrogenase activity was found after the mycotrophic grass *L. rigidum*, but differences between plants were lost after soil disturbance.

4 | DISCUSSION

The level of mycotrophy shown by the plants used in the experiment was confirmed, with *O. compressus* and *L. rigidum* being highly mycotrophic, whereas there were no arbuscular mycorrhizal fungi (AMF) colonized the roots of *S. gallica*. These results are consistent with those of Brito et al. (2014) and Alho et al. (2015).

The rhizosphere of mycorrhizal plants is considered to be microbiologically and biochemically more active than that of non-mycorrhizal plants owing to greater

deposition of carbohydrates from mycorrhizal roots to the soil (Andrade et al., 1998). In our study, following mycotrophic plants, general indicators of soil microbial activity, such as SBR, MBC, and dehydrogenase enzyme activity were significantly higher when compared with the non-mycotrophic. The greater values of SBR observed after *O. compressus* in the after-plant sampling are certainly associated with its greater AMF colonization but also with the microbial activity of rhizobia in its root nodules. Leguminous plants possess a greater provision of more readily decomposable materials which stimulate soil microorganisms that in turn may enhance biological activity in the rhizosphere (Koné et al., 2008). *L. rigidum* has an extensive root system with abundant root hairs (Caradus, 1980) and those attributes may result in the exudation of large quantities of organic compounds and thereby favour an increase of microbial biomass (Rocha et al., 2016) and the great values of MBC or DHA observed.

The root microbiome of plants grown in the same soil has been found to differ between plant species, thus the microbial community in the rhizosphere of mycotrophic plants is likely to differ from that associated with non-mycotrophic ones (Haldar & Sengupta, 2017). Our results showed a decreased count of Mn oxidizers after a legume when compared with the non-mycotrophic plant growth. Nogueira et al. (2007) also found a smaller number of CFU of Mn-oxidizing bacteria compared with non-mycorrhizal plants in their study and highlighted that the Mn availability in legume plants seemed to be driven by the balance among Mn-oxidizing and Mn-reducing bacteria associated with AMF in the rhizosphere (Nogueira & Cardoso, 2002). Despite the fact that when grown in this very same soil *O. Compressus* and *L. rigidum* harbour different AMF communities in their roots (Brígido et al., 2017), the count of most microbial functional groups and specific enzymatic activities did not show significant differences between the two mycotrophic plants. The presence of unculturable or recalcitrant organisms to the generalist culture media used (Hill et al., 2000) may have influenced these results and further phylogenetic analysis of microbiome changes associated with the studied treatments would be of interest.

Cropping systems strongly affect the soil biological activity, mainly by the choice of the plants cultivated, but the tillage regime adopted could also be a factor (Degrune et al., 2017). Soil disturbance, such as caused by tillage, can affect the soil microbiome and the biological processes they mediate, by changing soil aggregation, which in turn can modify water content and aeration, leading to modifications in soil function, stability and resilience (Smith & Collins, 2007). The mycorrhizosphere is a complex and metabolically active environment where the ERM influences soil bacterial communities and lead to a potential

functional complementarity (Linderman, 2008). The synergistic activity between the AMF and their associated microbiota is reflected in the complex network of interactions between them and their host plants (Mansfeld-Giese et al., 2002). In our study, a key effect associated with soil disturbance was the disruption of ERM and the loss of a particular habitat for many soil microbes, specifically P solubilizers, in addition with a great decrease in MBC and dehydrogenase activity. Many studies have shown that the tillage decreases the soil microbial biomass and enzymatic activity (Adetunji et al., 2017; Francioli et al., 2014; Laudicina et al., 2011; Madejón et al., 2009; Martin-Lammerding et al., 2013; Vazquez et al., 2017).

Although the negative effect observed for the main indicators of microbial activity caused by soil disturbance was widespread across all plants studied, its impact was not equally devastating for mycotrophic or non-mycotrophic plants nor for some of the functional microbial groups or specific enzyme activities. For mycotrophic plants, the count of P solubilizers, SBR, DHA and all the specific enzyme activities were more severely impacted by soil disturbance than for the non-mycotrophic plant. Such results confirm the importance of an intact ERM as a niche for soil microbial community persistence and activity. This was particularly important for the P solubilizer community, as also confirmed by the decrease in phosphatase activity. The solubilization of organic phosphate in the soil is linked to its microbiome and solubilization is closely associated with pH reduction and chelation that occurs owing to the release of organic and inorganic acids produced by bacteria and fungi metabolism (Kalayu, 2019). In that process, AMF play a key role by improving plant growth promoting bacteria known to be involved in P transformations and shown to live associated with the ERM (Taktek et al., 2015). This could explain the great number of P solubilizers among the mycotrophic plants in the soil samples collected after plant growth.

Irrespectively of plant mycotrophy, after soil disturbance a great decrease of general indicators of soil microbial activity (SBR, MBC and dehydrogenase activity) was noted in which all the differences previously observed between plants were lost and the activity came back to the range of values observed when no plants were growing in the soil. Specifically, in the case of dehydrogenase, tillage is known to strongly affect the activity of this enzyme by decreasing it over time owing to a rapid mineralization of SOM and subsequent decrease of oxidative biological activity (Malik et al., 2013). Increases in SBR during the first day of soil disturbance were reported by Kainiemi (2014) and after 1 week the rate decreased to base-rate. In the agronomic context of Mediterranean temperature conditions, these results

show the negative impact of tillage practices, which often leads to SOM impoverishment.

Mycorrhizal colonization facilitates plant growth when facing Mn toxicity stress (Brito et al., 2014; Faria et al., 2021). Keeping in mind the high levels of Mn in this soil and the fact *S. gallica* is a non-mycotrophic plant, its interaction with the soil microbiome is certainly distinct from the mycotrophic plants and the significantly large metabolic quotient (qCO_2) observed for this plant after soil disturbance reflects the differences in accessibility of C substrates, changes in metabolic rates (SBR and MBC) and changes in microbial community composition. The qCO_2 is usually low when the environment is more stable, or high when a stress occurs (Gajda, 2008; Guimarães et al., 2017) suggesting that the mineralization process after soil disturbance in *S. gallica* is different from the mycotrophic plants. Even though functional groups of culturable microorganisms or β-glucosidase activity did not change much under these circumstances, phosphatase and arylsulfatase activity significantly increased under S. *gallica* after soil disturbance. Soil enzymes are directly related to soil available nutrients and this could be reflected in different rates of SOM mineralization over time (Deng et al., 2019; Zarea et al., 2011). All together these elements indicate a different microbiome associated with its roots, also partially influenced by soil disturbance but in different ways from the mycotrophic plants.

Counts of fungi and ammonifiers were greater after the growth of all the plants and the latter significantly increased after soil disturbance. An increased count of Mn oxidizers bacteria after soil disturbance was also observed in our study. These results may have been associated with a decrease of redox potential observed after disturbance in the results of dehydrogenase activity and soil respiration. As suggested by Marschner and Timonen (2005), when the redox potential decreases, nitrate is used by microorganisms as an alternative electron acceptor, followed by manganese oxides. Sparrow and Uren (2014) found that even small changes in water potential, which in turn could be influenced by tillage practice, can shift this balance in favour of the soil manganese oxidation process.

In addition, S oxidizers were not influenced by any of the treatments, despite the changes observed in arylsulfatase activity. The SO_4 is the main source of S plant uptake, and it could be obtained by elemental S oxidation by S oxidizers bacteria or mineralization of the S organic forms present in the soil (Klose & Tabatabai, 1999). However, the methodology applied seemed to be unable to assess the results among the treatments imposed and therefore no significant differences were observed between the treatments. In another perspective, the S mineralization process mediated by arylsulphatase is the main process of S bioavailability to plants since the S organic forms constitute a major part of S content in soils (Gajda et al., 2013). Thus, our results of arylsulphatase activity showed clear differences in SOM mineralization associated with mycotrophic and non-mycotrophic plants.

5 | CONCLUSIONS

Plant growth, regardless of its level of mycotrophy, increased all the soil microbial parameters, sometimes considerably. For example, the activity of dehydrogenase more than tripled after plant growth when compared with the original bulk soil. Considering dehydrogenase as a key enzyme to evaluate general microbial activity, it unquestionably illustrates the importance of plants for soil microbial activity.

ERM and its specific hyphae-associated microbiome have been regarded as providing a differential biological dynamic. Our results confirmed the greater microbial activity associated with mycotrophic plants, with values of almost all microbial parameters increasing. In addition, the biological activity and functional profile of microbiota associated with mycotrophic and non-mycotrophic plant roots are differentially affected by the stress induced by soil disturbance. Significantly, metabolic differences between grasses and legumes were highlighted, with differences between these families being found in SBR, MBC, dehydrogenase and Mn oxidizers.

Biological activity and the functional profile of microbiota associated with mycotrophic and non-mycotrophic plant roots are differentially affected by the stress induced by soil disturbance. The bacterial population associated with the ERM of mycotrophic plants was very diverse, and the effect of ERM disruption resulting from soil disturbance was to decrease phosphatase activity and P solubilizers. In contrast, the count of P solubilizers associated with non-mycotrophic plants did not change and phosphatase activity increased following soil disturbance, suggesting different strategies for P acquisition by these plants. The specific results for enzyme activity of arylsulfatase, phosphatase and β-glucosidase activity together with the counts of P solubilizers and Mn oxidizers reflect the differential effects of soil disturbance on mycotrophic and non-mycotrophic plants.

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