

# Effects induced by living mulch on rhizosphere interactions in organic artichoke: The cultivar's adaptive strategy

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## Abstract

The plant root apparatus and the surrounding micro-environment is strongly influenced by specific abiotic and biotic conditions which occur in the plant rhizosphere system. The hypothesis of the reported research was that, in an organically managed horticultural system, the use of living mulch (LM) promotes the arbuscular mycorrhizal fungi (AMF) colonization among neighboring roots, because of the coexistence of different plants roots in confined soil spaces. This effect determines nutrient uptake optimization, although roots belong to different plant species. In the reported 2-yr field experiment (2012–2013), two Italian artichoke cultivars [*Cynara cardunculus* L. var. *scolymus* (L.), *Jesino* cv. and *Mazzaferrata* cv.] were intercropped with a LM mixture of plant species and compared with a no LM control. Every year, the effect of LM on artichoke root morphology and AMF colonization was evaluated by scanning electron microscopy, in order to assess abiotic and biotic rhizosphere interactions, as affected by artichoke cultivars. Also the artichoke yield, the soil available phosphorus (P) and rhizosphere P were determined. Results showed that the LM did not reduce yield of both the artichoke cultivars, when compared with the no LM ones. Furthermore, LM has: (i) induced structural changes in artichoke roots by proliferation of root hairs resulting in an increase of effective absorbing surface; (ii) promoted the rhizosphere mycorrhizal infection which improved P uptake. The modified rhizosphere interactions were found to be cultivar-dependent, being recorded only in *Jesino* artichoke.

**Key words:** cover crop, AMF, vegetable, root hairs, SEM, rhizosphere P

## Introduction

The need for redesigning organic horticulture strategy on the basis of agroecological principles has been acknowledged (Altieri and Rosset, 1996) to promote the positive interaction among plant species and among plants–soil microorganisms. The environmental sustainability of organic vegetable production is higher than in conventional systems, not only for the improvement of resource recycling and the related reduction of pollutants, but also in relation to a high level of biodiversity conservation (Raviv, 2010).

In a 7-yr rotation trial, the effect of low-input (organic, bio-dynamic) and high-input (conventional) farming managements on arbuscular mycorrhizal fungi (AMF) infection of the cash crop roots was evaluated (Mäder et al., 2000), observing an increased AMF colonization for the

organic management. This finding was interpreted as a consequence of the reduced external inputs in organic farming systems corresponding to a lower soil nutrient availability for the crop, so as to activate positive rhizosphere symbiotic interactions.

An increased interrelationship among crop roots and soil mycorrhizal fungi could potentially improve nutrient uptake, particularly phosphorus (P) and, indirectly, nitrogen (N) by the cash crop (Smith and Read, 2009). This is particularly relevant in organic systems, where the use of natural mulches can promote appropriate fungal symbiotic associations, thus ensuring the proper water and nutrient uptake (Derkowska et al., 2008; Ciaccia et al., 2015). At the moment, the role of plant biodiversity on AMF root colonization (Burrows and Pfleger, 2002) or, conversely, the ecological function of mycorrhizal fungal diversity on the maintenance of plant biodiversity (van

der Heijden et al., 1998) is not completely clarified. Anyway, a sustainable agricultural system, characterized by a high biodiversity, should guarantee both the crop yield and the soil quality on long terms (Mazzoncini et al., 2010; Bårberi, 2015), also by taking advantage of the cultivar adaptability for increasing water and nutrient use efficiency (Campanelli and Canali, 2012). The use of living mulch (LM) (i.e., cover crops intercropped within the cash crop) is a good chance to ensure plant biodiversity, offering a series of agroecological services such as the increase or maintenance of crop yield (Masiunas, 1998; Kremen and Miles, 2012), the reduction of water and nutrient loss (Swenson et al., 2004; Kristensen et al., 2014), the weed suppressive potential (Baumann et al., 2000) and the control of plant pest and pathogens (Kołota and Adamczewska-Sowińska, 2013; Burgio et al., 2014). On the other hand, it is well known that AMF can simultaneously colonize the roots of multiple plants to form a shared mycorrhizal network, through which the nutrients can be transferred from one plant to another (Cheng and Baumgartner, 2005).

The effect of LM on rhizosphere interactions represents one of the most interesting black-boxes to disclose. In a multi-crop system, as a designed-organic horticultural one, the vegetable crop, the living much and weeds share the belowground space: each plant root apparatus and the surrounding micro-environment are strongly influenced by specific abiotic and biotic conditions which occur in such a competitive rhizosphere system. The co-presence of different plant species in confined spaces could affect the root development and, consequently, promote or inhibit soil-borne plant-microbe interactions, inducing positive or negative effects also on crop mineral uptake and yield. In cereals, mulched with different organic residues, mycorrhizal roots developed in such a way to ensure the highest water and nutrient uptake (Kaldorf and Ludwig-Müller, 2000). This was achieved by improving their size and increasing the number of lateral roots and root tips, thus overcoming the potential competition for limited soil resources (Al-Karaki, 2004). A similar behavior was also recorded in citrus trees, which reacted to alternative organic amendments by increasing the root mucilage production and roots' lateral buds (Trincherà et al., 2015).

The present work is referred to a typical Mediterranean vegetable crop, the artichoke [*Cynara cardunculus* L. var. *scolymus* (L.)], organically cultivated in the Centre of Italy, taking into account two different local cultivars. The hypothesis of the research is based on the assumption that a LM mixture of plant species, intercropped with the artichoke, is able to modify abiotic and biotic rhizosphere interactions for sustaining crop yield by: (i) modifying structural root morphology to increase the effective rhizosphere absorbing surface; (ii) promoting biotic plant-microorganism associations, in particular the root colonization by AMF.

## Materials and Methods

### Experimental field trial

In order to verify the hypothesis, a 2-yr (2012–2013) field experiment was implemented in the MONsampolo VEgetable organic Long-Term field Experiment (MOVE LTE) located in the CREA-Research unit for vegetable production (CREA-ORA) of Monsampolo del Tronto, in central Italy (42°53'N, 13°48'E). The climate at the site is thermo-mediterranean, with annual temperatures which range between 0°C in winter and 40°C in summer. The rainfall distribution throughout the year is uneven, the autumn season being most predominant. The soil at the field trial was a Typic Calcixerepts fine-loamy, mixed thermic one (USDA, 1996).

Living mulch was used for organically managing the weeds growth in a multi-annual artichoke (*C. cardunculus* var. *scolymus*) field, in relation to two artichoke cultivars: *Mazzaferrata* (Ma) and *Jesino* (Je), both derived from a local (medium-Adriatic) germplasm, the first one being characterized by the highest productivity (Ficcadenti et al., 2013). In 2012 and 2013, artichoke was intercropped in a randomized three block-designed system with a suitably selected mix of plant species (*Trifolium incarnatum* L., *Vicia villosa* L., *Vicia faba* L. var. *minor*, *Coriandrum sativum* L., *Fagopyrum esculentum*, *Alyssum* spp., *Pisum sativum* L., *Brassica rapa* L., *Phacelia tanacetifolia* Benth). In the first year of experiment, LM was broad sowed on November 2, 2011, while in the second one on February 21, 2013. In both years, LM was terminated at the end of June. Two factors were considered: the LM and the artichoke cultivar. The first factor had two levels: no living mulch (control, no LM) and unweeded LM during the whole cropping cycle (LM).

### Artichoke rhizosphere interactions

For both 2012 and 2013, in spring, at harvest and before LM termination, artichoke root systems were collected from the field using stainless steel cylinders of 10 cm diameter and 25 cm length, put at the distance of 15 cm from the artichoke plant stem. Two plants per plot were sampled, with a total of 2 plants × 2 LM treatments × 2 cultivars × 3 blocks = 24 artichoke root samples. Within 24 h, roots were separated from the soil by washing the sampled material under fresh water in a sieve of 0.5 mm mesh, which were divided into first, second and third-order lateral roots for further analyses.

To evaluate the effect of LM on root morphology and the arbuscular mycorrhizal colonization, roots fragments were collected from each considered artichoke plant. Third-order fine lateral roots of five artichoke (diameter < 2 mm) were cut with a razor blade from 5 to 15 mm from the root tip, for assessing the potential effect induced by LM on root cortex cells, root hairs (rh), AMF hyphae (hyp) colonization. The selected fresh root fragments were observed by scanning electron microscopy

(SEM) (Microscope Zeiss—EVO MA10) under variable pressure equipped with a tungsten or a LaB<sub>6</sub> electron sources, using both the secondary electrons detector (SEM\_VPSE) and the back-scattered electrons detector (SEM\_CZ-BSE). The applied variable pressure mode (at 20–25 kV EHT/10 Pa chamber pressure) prevented surface damage of such biological and non-conductive samples, giving a high-resolution image without any prior sample preparation. SEM was also implemented by the Beam Sleeve technology, able to extend the vacuum column to 2 mM of the specimen in order to improve contrast and analytical accuracy. The use of the two alternative detectors, VPSE and CZ-BSE, allowed us to obtain different information: the first one (equipped with W electrons source) inspected the surface topography of the root tissues, giving a resolution which allowed us to detect some biological microstructure types; the second one (equipped with LaB<sub>6</sub> electrons source, which guaranteed a very high brilliance, through optimizing the performances of back-scattered electron microscopy), differentiated the materials in relation to the light contrast, that means to the different average atomic weight, thus informing on the composition of the biological object under study.

The quantification of root mycorrhization (AMF) was performed after root staining, carried out by immersing them in a stain solution of 0.05% (w/v) methyl blue in lacto-glycerol (1:1:1 lactic acid, glycerol and water) for 1 min, and then destained in bi-distilled water for 1 min more (Grace and Stribely, 1991). A total of 10 × 1 cm root pieces (third-order lateral fine roots) per plant were selected at random from the stained root fragments and placed on a microscope slide. The root fragments, mounted in a drop of glycerol, were observed using an optical stereo-microscope (Nikon SMZ-U). Microscopic images were taken using a digital camera (Nikon Coolpix 8400). The intensity of AMF colonization in the root system (M%) was evaluated as symbiotic parameter (Trouvelot *et al.*, 1986), based on visual characteristics, using mycorrhizal infection scores in classes from 0 to 5:

$$M\% = (95n_5 + 70n_4 + 30n_3 + 5n_2 + n_1) / \text{total number of observed fragments}$$

where  $n_5$  is the number of fragments rated 5 (i.e., with the maximum mycorrhizal colonization frequency),  $n_4$  is the number of fragments rated 4 and so on.

### Artichoke yield, soil available P and rhizosphere soil P

In order to evaluate the effect of LM on artichoke production, at the end of each harvest period (2012, 2013), artichoke yield was measured (Mg ha<sup>-1</sup>), calculated as the sum of first, second and third-order artichoke heads.

On the basis of the recognized relationship between plant root mycorrhization and P availability in soil, in both 2012 and 2013, bulk soil available P (P<sub>av</sub>, mg K g<sup>-1</sup>)

was determined at artichoke harvest, by sampling soil at 0–30 cm depth, extraction according to Olsen method (Olsen *et al.*, 1954) and following analysis by continual flow colorimeter.

Only in 2013, at artichoke harvest, rhizosphere soil P (P<sub>rhizo</sub>, mg K g<sup>-1</sup>) was checked to evaluate if the available P was influenced by the mycorrhizal root colonization within the soil-root zone. The artichoke roots, contained in each stainless steel cylinder, were shaken softly by hand to remove the rhizosphere soil. This soil adhering to the surroundings of the roots was collected, sieved in a 1 mm mesh to remove root hairs (Furubayashi *et al.*, 2003), extracted according to Olsen method and then analyzed for P<sub>rhizo</sub> by continual flow colorimeter.

All the plant and soil laboratory tests were carried out in triplicate in order to control intra-laboratory variability.

### Statistical analysis

After calculation of M%, results were evaluated by univariate analysis of variance (ANOVA), considering LM presence and cultivar as factors. Mean comparison was carried out according to least square difference (LSD) and Duncan Multiple Range Tests (DMRT), at  $p \leq 0.05$  probability level.

Artichoke yield, P<sub>av</sub> and P<sub>rhizo</sub> were analyzed by univariate ANOVA, considering year, LM presence and cultivar as factors. Mean comparison was carried out according to LSD and the DMRT, at  $p \leq 0.05$  probability level, for two and more than two comparisons, respectively.

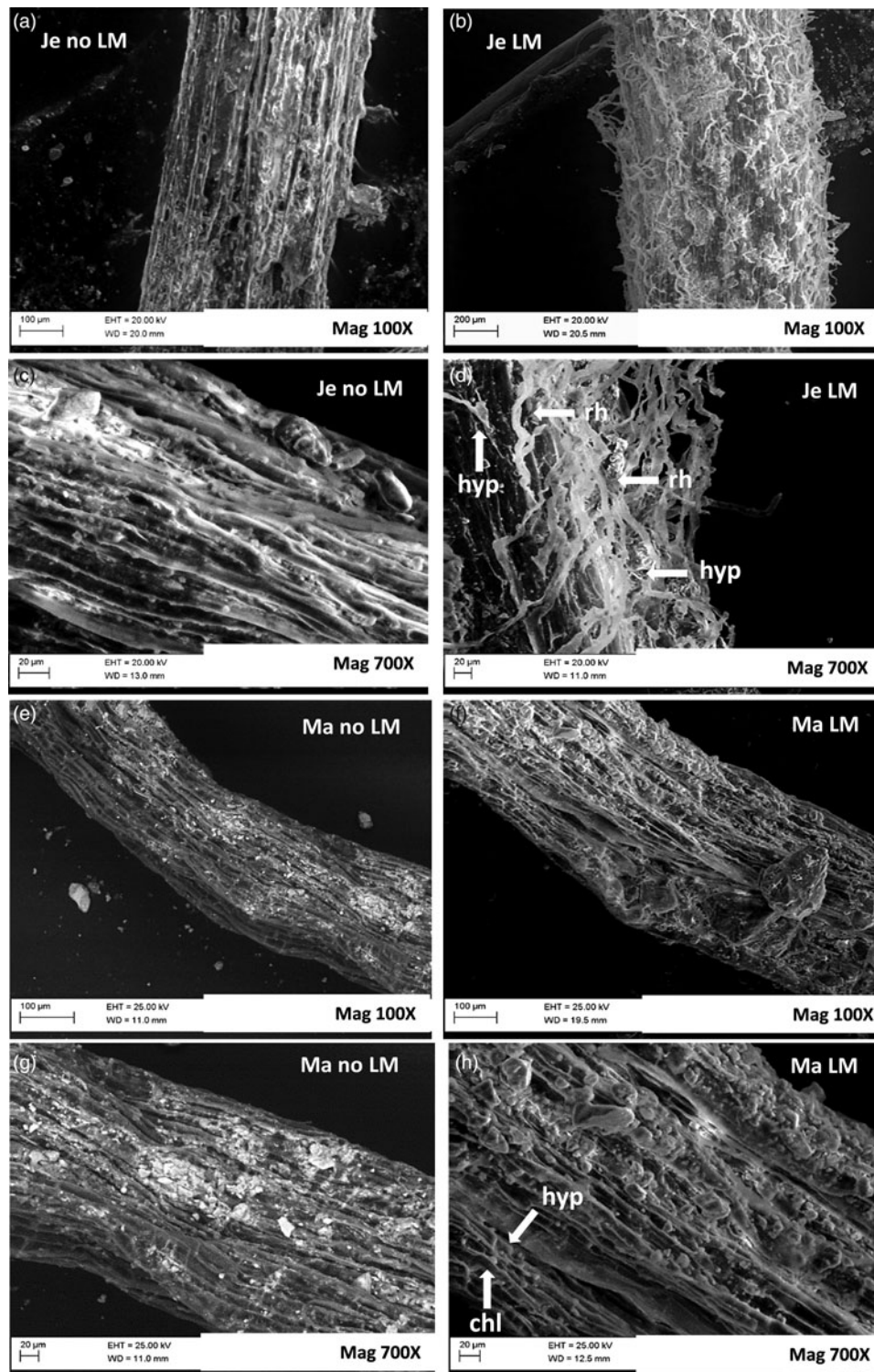
The elaboration was performed using the SPSS 16.0 package.

## Results

### Evaluation of rhizosphere interactions

The Je (Fig. 1A–D) and the Ma (Fig. 1E–H) artichoke cultivars showed different behavior in relation to the presence of LM: particularly, in the Je roots the LM mix induced the proliferation of many root hairs (Fig. 1B, D, rh), that were not detectable in the no Je LM roots (Fig. 1A, C). This finding was not observed in Ma cultivar, where both the no LM (Fig. 1E, G) and the LM (Fig. 1F, H) artichoke roots did not show rh proliferation. Some distributive mycorrhizal hyphae were detected on the cortex surface in LM roots in both Je and Ma cultivars (Fig. 1D, H), but not in the no LM treatments (Fig. 1C, G).

The intensity of root colonization by AMF, calculated in relation to the artichoke cultivar and LM treatments, are reported in Table 1 and Figure 2 (X–W), respectively. The Ma cultivar was not affected by LM in relation to root AMF infection (21% in no LM and 23% in LM), while a significant ( $p < 0.01$ ) effect of LM was recorded in Je, where the intensity of root colonization increased from 15% in no LM to 43% in LM artichoke.



**Figure 1.** Effect of LM on Je and Ma artichoke fine root (third order lateral roots). Images are referred to 100× and 700× magnifications by SEM. White arrows indicate: rh, root hairs; hyp, mycorrhizal hyphae; chl, chlamydospores (Ma: cv. *Mazzaferrata*; Je: cv. *Jesino*).

Microscopic observation of AMF colonization in Je and Ma roots under LM or no LM treatments by SEM\_CZ-BSE is reported in Figure 2. The Je showed the mycorrhizal colonization both in no LM root (Fig. 2A) and in LM on (Fig. 2B), but with a relevant

increase of AMF colonization under LM mix. The cortex cells of no Je LM roots supported the presence of mycorrhizal fungi, evidenced by the presence of extra-cellular hyphae (Fig. 2C). Otherwise, in Je LM roots, the AMF external, intercellular (localized in air channels)

**Table 1.** Intensity of AMF colonization (M% of the total root length) and rhizosphere P at artichoke harvest ( $P_{\text{rhizo}}$  in  $\text{mg kg}^{-1}$ ), as affected by LM for Je and Ma artichoke cultivars; data referred to 2013.

Artichoke cultivar	Intensity of AMF colonization (M%)		$P_{\text{rhizo}}$ ( $\text{mg kg}^{-1}$ )	
	No LM	LM	No LM	LM
Je	15 b	43 a	27.2 a	13.4 b
Ma	21 b	23 b	16.8 b	15.8 b

Mean values followed by a different letter within each cultivar are significantly different according to LSD at the reported probability level ( $p \leq 0.01$ ). Je: cv. Jesino; Ma: cv. Mazzaferatta.

or intracellular (ramified within the cortex cells cytoplasm) hyphae, together with the additional profusion of AMF spores (spr) (Fig. 2D) attested the promoting effect of LM on AMF root colonization. Moreover, Je cultivar showed the presence of extra-hyphae in LM treatment (Fig. 2X–Y), not found in the no LM one (Fig. 2C–D). This finding corresponded to the quantitative information reported in Table 1. The Ma artichoke cultivar showed a different behavior: the entity of mycorrhization was similar in the LM (Fig. 2, F) and no LM roots (Fig. 2E), with the predominance of external AMF hyphae (Fig. 2Z–W). Again, the quantitative data were confirmed by roots visual inspection, since no relevant differences were recorded in cortex root cells colonization by AMF, even if some intercellular hyphae were recorded in Ma LM (Fig. 2H), not observed in Ma no LM (Fig. 2G).

### Artichoke yield, P soil availability and rhizosphere soil P

Results for artichoke yield are reported in Table 2. In 2012 and 2013, no significant differences were recorded among the artichoke yields, even though the highest mean value was reached in 2013. As far as the LM effect is concerned, the artichoke yield was not affected by this factor. On the other hand, the Ma produced significantly higher yield ( $5.8 \text{ Mg ha}^{-1}$ ) than the Je one ( $4.1 \text{ Mg ha}^{-1}$ ), as expected. There was no significant two- or three-way interaction.

In Figure 3, results of the bulk soil  $P_{\text{av}}$ , measured in 2012 and 2013 at harvest, are reported. In both the years,  $P_{\text{av}}$  was significantly lower in LM with respect to no LM for both the two tested cultivars. In 2012, the mean  $P_{\text{av}}$  was the lowest when compared with that recorded in 2013 ( $9.0$  and  $20.4 \text{ mg kg}^{-1}$ , respectively).

In 2013, the rhizosphere soil  $P_{\text{rhizo}}$  of Je LM at artichoke harvest (Table 1) was much higher than that of the Je no LM, similarly to what happened for soil  $P_{\text{av}}$ ; conversely, no significant differences were observed between Ma no LM and Ma LM.

## Discussion

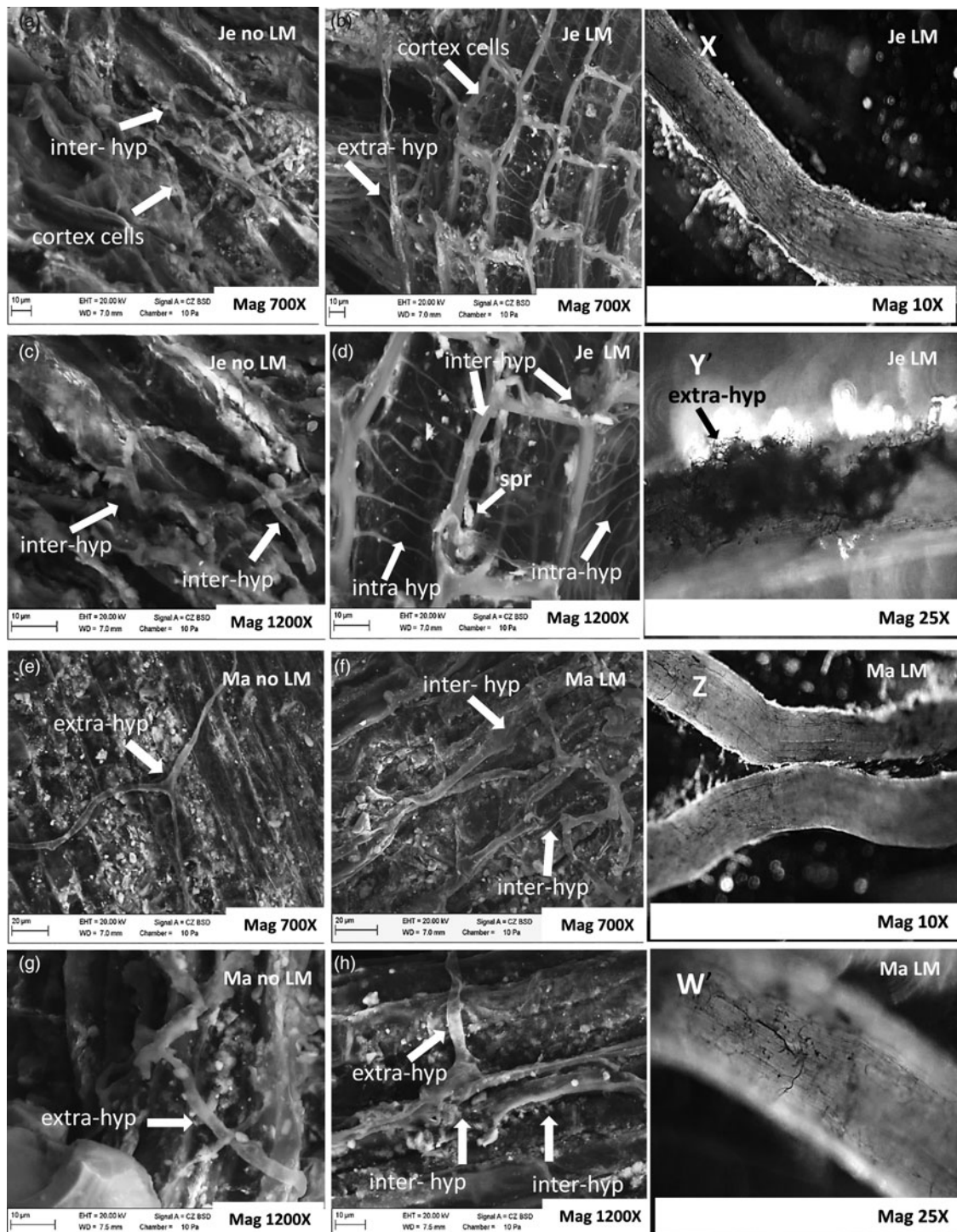
Climatic conditions did not affect artichoke performances, since crop total fresh yield was not statistically

different between the two tested years. In Central Italy, since the main artichoke growing season is the autumn–winter, rainfall as well as minimum temperature represent the main limiting factors of crop yield. Therefore, observed outcomes were likely related to the standard climatic conditions (data not reported) that occurred during the studied period.

Many authors reported how the use of LM on different horticultural crops reduces the crop yield (Hiltbrunner *et al.*, 2007; Chase and Mbuya, 2008). Conversely, Canali *et al.* (2015) observed that the introduction and the opportune management of LM (i.e., the presence of legumes), the high biodiversity of the plant species and the proper sowing-termination time, allowed avoidance of nutrient and water competition among different co-existing plant species, determining similar yield between the living mulched and the sole crop. Accordingly, in our experiment LM did not affect the artichoke yield (Table 2).

Artichoke yield was instead strongly influenced by the cultivar (Table 2), confirming Je as a low-yield artichoke cultivar, characterized by small plants and less lateral shoots than other ones, such as the Ma (Ciancolini *et al.*, 2013). Otherwise, the Je LM showed the most relevant changes on root apparatus. The co-presence of LM mix and artichoke created a belowground micro-environment, within which the rhizosphere interactions, both at anatomical and biological levels, played a key role, as already observed in such organic agroecosystems (Verbruggen *et al.*, 2010). The proliferation of rh observed in Je roots (Fig. 1) in presence of LM represents the first, effective adaptive strategy by which the Je artichoke attempted to optimize the soil water and nutrient uptake by increasing the root adsorbing surface (Hill *et al.*, 2010).

Even if the presence of rh can be associated with a reduced mycorrhization (Plaxton and Lambers, 2015), in our experiment the Je LM roots showed a higher arbuscular mycorrhizal colonization (Fig. 2) with respect to the no LM one (Mäder *et al.*, 2000). This finding was also confirmed by the calculated intensity of mycorrhization (Table 1). Although it is well known that the intrinsic metabolic cost in mycorrhizal roots is higher than in non-mycorrhizal ones, due to a substantially higher cell respiration (Shachar-Hill *et al.*, 1995; Douds *et al.*, 2000), in Je LM



**Figure 2.** Effect of LM on mycorrhization of Je and Ma artichoke root cortex cells (fine, third-order lateral roots). Images A–H are referred to 700 $\times$  and 1200 $\times$  magnifications by SEM, while images X–W to 10 $\times$  and 25 $\times$  magnifications by stereo-microscope. Arrows indicate: cortex cells; extra-, inter- and intra-hyp = mycorrhizal hyphae; spr = spores (Ma: cv. *Mazzaferrata*; Je: cv. *Jesino*).

the potential benefits achievable through root AMF infection exceeded this metabolic disadvantage. Actually, the Je cultivar showed a mycorrhization well developed within the cortical cells, with numerous and widespread inter- and intra-radical hyphae, these last ones not present in the no LM artichoke. The AM colonization found in the Je LM roots potentially increased their ability to explore

the soil: the promotion of the intra and external radical hyphal growth (intra-hyp and extra-hyp; Fig. 2) is a clear cultivar-dependent mechanism, by which several benefits are conferred through abiotic and biotic interactions in the rhizosphere for enhancing nutrient uptake. Given the role of intra-radical hyphae in AMF carbohydrate (i.e., hesose and glycogen) metabolism and lipid storage (Bago

**Table 2.** Artichoke yield (in Mg ha<sup>-1</sup>), calculated as the sum of first, second and third-order heads; data referred to 2012–2013.

Factor	Level	Artichoke heads production Total fresh yield (Mg ha <sup>-1</sup> )
Year	2012	4.3
	2013	5.6
LM		n.s.
	+	4.9
	-	5.0
		n.s.
CV	Je	4.1
	Ma	5.8
		*

The mean values in each column followed by a different letter are significantly different according to LSD at the reported probability level. n.s., not significant. \* $p \leq 0.05$ . Je: Jesino; Ma: Mazzaferrata; +: LM; -: no LM; CV: cultivar.

*et al.*, 2000), their proliferation in Je LM roots attests the increase of C translocation in favor of the mycorrhizal fungi and the consequent effectiveness of plant–fungi symbioses in promoting the nutrient uptake by the Je artichoke cultivar in presence of LM.

On the other hand, the AMF are also able to alter water relationships of plants, improving their tolerance to drought (Birhane *et al.*, 2012): the presence of LM mix may have induced the Je artichoke to compete for water more than in the presence of the sole weeds, in such a way as to increase the active absorbing root surface *i.e.*, by multiplying the rh and building an efficient and mycorrhizal network.

Regarding the Ma artichoke, Jeffries *et al.* (2003) and Tawarayama (2003) recognized the role of root AMF infection in alleviating plant abiotic stresses, depending on plant cultivars. The same M% recorded in Ma LM and Ma no LM (Table 1) was probably due to the characteristics of this cultivar, less affected by the aboveground–belowground interrelationships established by LM mix with respect to the Je one.

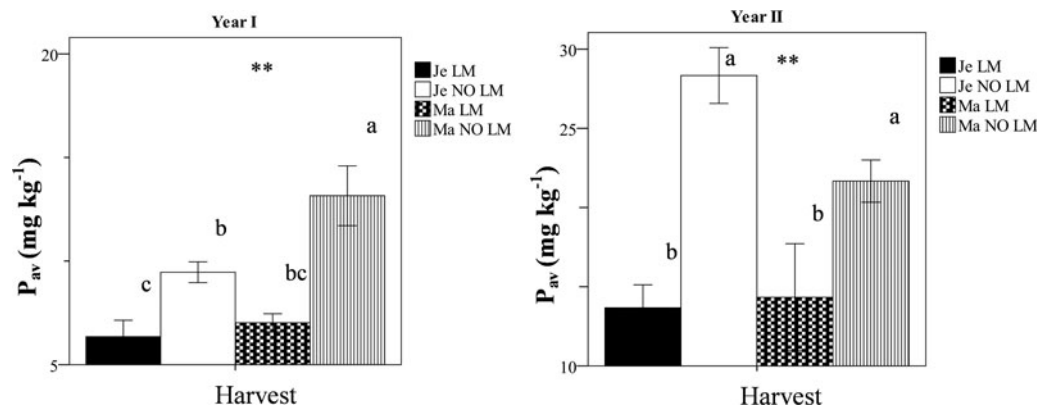
In order to explain in more depth the building up of the recorded abiotic (increased rh) and biotic (root-AM colonization) interactions in the Je artichoke, it should be remarked that the P<sub>av</sub> in Je LM and Ma LM bulk soils was lower with respect to that of the no LM ones (Fig. 3), indicating that, after both the 2-yr cropping cycles, the bioavailability of soil P decreased in the artichoke–LM systems with respect to the control.

Differently, the P<sub>rhizo</sub> (Table 1) had a different behavior as far as the two cultivars were concerned. In particular, it was the same in Ma LM and Ma no LM, while it was again significantly lower in Je LM with respect to the no LM, that means in correspondence to the increase of root hairs development in Je LM roots. This is in accordance to Keyes *et al.* (2013), who verified that the P depletion achieved by the root hairs is generally more local

than that achieved by roots, and it is heavily dependent from root hairs orientation and the related contact with soil aggregates. Moreover, the improved AMF development may have favored the ability of the plants to advantageously utilize the organic forms of N and P (Kahiluoto and Vestberg, 1998). All these considerations indicate that, in presence of LM mix, the attitude of Je artichoke roots in utilizing soil P was more effective than that of the Ma ones, so much to significantly reduce the residual bulk soil P<sub>av</sub> at the end of the cropping cycle, corresponding to a strong decrease of rhizosphere P in the Je root-zone.

Actually, since the bulk soil P<sub>av</sub> was the lowest under LM mix after the cropping cycles, it can be hypothesized that, in the no LM systems, the P was mainly present in the soil in mineral forms (highest P<sub>av</sub>). On the contrary, under LM, the P was immobilized by the AMF, which proliferated in such a specifically biodiverse LM system: subsequently, the AMF transferred the P to the Je artichoke and to the LM mix, by efficiently exploring the soil through the symbiotic association within the rhizosphere. The P uptake of Je LM artichoke heads (1st + 2nd + 3rd orders) showed a tendency to increase (+26% on the 2012–2013 mean value; data not shown). These results are in line with those obtained by Liu *et al.* (2003), who observed that, in a field trial on maize, the extra-radical AMF hyphae density was negatively correlated with soil extractable P. This suggests that, when soil P availability and host plant genotype are favorable to mycorrhizal development, the soil extractable P is reduced due to AMF-enhanced plant P uptake. In our experiment, Je LM gave the highest M%, with a proliferation of AMF extra-radical hyphae and the lowest P<sub>av</sub> and P<sub>rhizo</sub> (Table 1), fully confirming the results obtained by Liu *et al.* (2003).

It is important to underline the function of LM in adjusting the already mentioned agroecological adaptive capacity of the Je artichoke. We can hypothesize that, in such a designed living mulched agro-ecosystem, the ‘addressed’ plant biodiversity due to LM may have acted by promoting the recorded virtuous abiotic and biotic rhizosphere interactions among neighboring plants, this being a response to the LM of artichoke cultivar-dependent. Njeru *et al.* (2014) had already observed that, in organic corn production, the use of different cover crops, by increasing genetic and species diversity, provided a more favorable environment for mycorrhizal activity, promoting the mycorrhizal colonization and the following increase of corn early growth, independently from the corn genotype diversity. This means that the presence of cover crops induced both a biofunctionality effect and a functional biodiversity effect (Campanelli and Canali, 2012). In our experience, the LM expressed its role as ecological service crop by providing a functional biodiversity, in which the LM effect (*i.e.*, the biofunctionality) was actually linked to a specific artichoke cultivar, with the rhizosphere functionality able to ensure a most effective nutrient utilization pattern.



**Figure 3.** Soil available P ( $P_{av}$ ) at artichoke harvest (in  $\text{mg kg}^{-1}$ , data referred to 2012–2013). Mean values are significantly different, according to DMRT at the reported probability level (\*\*: significantly different at  $p \leq 0.01$ . Ma: cv. Mazzaferrata; Je: cv. Jesino. +: LM; -: no LM).

## Conclusions

In our designed agro-ecosystem, the used intercropped LM showed its agro-ecological function not only in relation to the potential effect on weed management, or the maintenance of soil water moisture and temperature, but also in promoting virtuous rhizosphere interactions, able to provide a different use of the available resources. The induction of changes in artichoke root morphology, such as the root hairs proliferation, is an effective strategy to guarantee an adequate nutrient supply to the crop, especially in a low-yield cultivar, potentially most responsive to external interference. On the other side, the increased artichoke mycorrhization, with the appearance of many extra-radical and intra-radical AMF hyphae recorded in Je LM, represents the best eco-physiological response to ensure the proper P availability to the crop, guaranteeing contemporary a good standard yield.

In conclusion, the used LM, when intercropped with a vegetable cultivar able to better meet with such management, demonstrated to be able to optimize the sharing of the rhizosphere by implementing the root morphology and promoting AMF infection, taking advantage from the selected plant biodiversity over the spontaneous, invasive species. This observed interactive mechanism may be considered an example of functional agrobiodiversity operating at crop species or habitat level, able to deliver a given agroecosystem service, thus consequently supporting sustainability.

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