

Impact of calcium associated to calcareous amendments on ectomycorrhizae in forests: A review

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Abstract

There is currently little information available on calcium-soil-ectomycorrhizal (ECM) interactions. However, for decades calcareous amendments have been made in forest soils subject to acid rain in Europe without any clear knowledge of their impact on ECMs and the roots of their host plants. We have therefore performed a review to assess the impact of Ca associated to calcareous amendments in the soil-ECM-host plant system, which has highlighted: (1) the influence of Ca and its salts (carbonates and oxalates) of biological origin in ECM fungal communities; (2) the impact of Ca on the growth patterns of the host tree roots; (3) the importance for the plants of the rock-eating processes associated to ECMs; and (4) the impact on ECMs of the soil Ca cycle (associated to the litterfall). A greater knowledge of the cause-effect relations between Ca and the soil-ECM-plant system in Mediterranean and temperate forests could have positive repercussions on reforestation projects, actions associated to soil management, the commercial production of ECMs, and the economy of different rural zones in these forest areas.

Keywords: Ectomycorrhizas, Mediterranean forests, temperate forests, acid rain, rock-eating

1. Introduction

Calcareous amendments have been widely used in agroforestry management both tropical and temperate reforestations. For several decades the forestry industry has intensified its use of calcareous amendments to counteract the impact of acid rain in several forests in north and central Europe, which have a great abundance of ECM

fungi. Several authors have warned of the lack of knowledge on the impact of the Ca associated to these calcareous amendments on the rhizosphere of these forests (Gronflaten *et al.*, 2005; Naramabuye *et al.*, 2008; Löfgren *et al.*, 2009; Lee *et al.*, 2011; Monfort-Salvador, 2013).

There are known to be negative interactions between excess soil Ca and the processes of chlorosis in plants. A number of researchers have studied this chlorosis in pines, firs, apple trees, vines, peanuts, dwarf beans, green beans, soy, lupins and other plants (García-Montero *et al.*, 2009). The abundance of finely divided calcium carbonate in the soil increases the levels of exchangeable Ca^{2+} and HCO_3^- and generates a basic pH, which precipitate P, B, Fe and Mn (Gaucher, 1971; Douchafour and Souchier, 1979; Follet *et al.*, 1981; Loué, 1986; Wild, 1992; Callot, 1999).

The most important effect of liming is increase of soil pH, moves out the Al^{3+} from the cationic exchange positions and hence reduces soil acidity, modify the Cationic and Anionic Exchange Capacity of the soils when they have and “variable change systems” (depending on the isoelectric point). However, calcareous amendments produce also reactions between the Ca^{2+} and P in the soil solution and in the soil colloids, and also cause secondary Ca carbonate precipitations. The degree of immobilisation of P by the Ca carbonate is inversely proportional to the size of its particles. Adding excess Ca^{2+} and HCO_3^- in the soil can cause Mg^{2+} deficiencies in plants (Follet *et al.*, 1981), and generate secondary calcites that also immobilise Mg^{2+} (Douchafour and Souchier, 1979).

Borja and Nilsen (2009) and Bakker *et al.* (2000) indicate that calcareous amendments may have a long-term impact on forest rhizospheres, as they have observed that the effects are evident 27 and 35 years after application, respectively. For this reason, more knowledge is also needed on the

impact of calcareous amendments on ECM fungi in the forest environment; this has been a matter of increasing concern in the scientific literature in recent decades (Erland and Söderström, 1990; Schneider and Zech, 1990; Antibus and Linkins, 1992; Huettl and Zoetl, 1993; Kreutzer, 1995; Jonsson *et al.*, 1999; Bakker *et al.*, 2000).

The present review aims to contribute to the knowledge of the interactions between Ca associated to calcareous amendments and soil-ECM-plant systems, and to assess the potential impact of liming on forest rhizospheres.

2. Effect of calcareous amendments on forest scale

2.1 Interaction between calcareous amendments and soil nutrients

After calcareous amendments, it has been observed that one of the main effect of the amendments is that they provoke an increase in soil pH (Borja and Nilsen, 2009; Rineau and Garbaye, 2009) (Table 1).

Calcareous amendments increase concentrations of Fe, K, Si Ca, Mg and Mn in the soil because of their previous presence in carbonate rocks that constitute the raw material of amendments (Table 1). Rineau and Garbaye (2009) and Rineau *et al.* (2010b) indicate that in the control plots in their study, the initial concentrations of Ca, Mg and Mn were low or very low. However, after calcareous amendments these three nutrients were the elements whose concentration had increased most. Rineau *et al.* (2010b) did not observe any change in Na, S and P concentrations after the amendments.

Rineau *et al.* (2010b) indicate that in some plots with acid soils of *Fagus sylvatica* L. mycorrhized with *Lactarius subdulcis* (Pers.: Fr.) S.F. Gray, calcareous amendments produced an increase in Al levels in the soil. Qian *et al.* (1998) made additions of sulphuric acid and calcareous amendments (obtained from dolomites) in formations of *Picea abies* (L.) Karst. These authors also observed high concentrations of Al in the soil, accompanied by high mycorrhizal

activity. In contrast, in several plots of *Pinus sylvestris* L. and *Fagus sylvatica* associated with ECM fungi, Rineau and Garbaye (2009) observed a decrease in soil Al concentrations, although they did not venture any explanation of these findings. However, it is necessary to note that the interactions between calcareous amendments and exchangeable Al are a complex question in soil chemistry, strongly dependent on soil genesis and local soil processes.

Table 1. Comparison of the effects of calcareous amendments on soil nutrients: (>) increased concentration; (<) decreased concentration; (=) the concentration remains the same (soil elements when the calcareous amendments occur)

Authors	Host species	ECM species	Ca	Mn	Al	Fe	Mg	K	Na	S	P	pH	N	Si	C
Rineau <i>et al.</i> (2010b)	<i>Fagus sylvatica</i>	<i>Lactarius subdulcis</i>	>	>	>	>	>	>	=	=	=				>
Rineau and Garbaye (2009)	<i>Fagus sylvatica</i> <i>Picea Abies</i>	-	>	>	<		>					>	<		<
Andersson, Jensé and Söderström (1996)	<i>Picea Abies</i> <i>Betula péndulas</i>	<i>Paxillus involutus</i> Batsch : Fr.	>		=		>				<	>			
Kjoller and Clemmensen (2009)	<i>Pinus sylvestris</i> and <i>Picea abies</i>	-			<							>			

Table 2. ECM species whose natural populations increase in response to calcareous amendments

Authors	ECM species	Host species
Kjoller and Clemmensen, (2009)	<i>Amphinema. Byssoides</i> (Pers.) J. Erikss.	Coniferous
Rineau and Garbaye (2009)	<i>Amanita rubescens</i> (Pers.: Fr.) S.F. Gray	<i>Fagus sylvatica</i> y <i>Picea</i> spp.
Kjoller and Clemmensen (2009)	<i>Atheliaceae</i> sp.	Coniferous
Rineau, et al. (2010a)	<i>Clavulina cristata</i> (Holmsk.) J. Schröt	<i>Fagus sylvatica</i> y <i>Picea</i> spp.
Jonsson et al. (1999)	<i>Cortinarius</i> sp.	<i>Picea abies</i>
Kjoller and Clemmensen (2009)	<i>Elaphomyces</i> sp	Coniferous
Jonsson et al. (1999)	<i>Hebeloma crustuliniforme</i> (Bulliard) Quélet	<i>Picea abies</i>
Rineau et al. (2010a)	<i>Lactarius subdulcis</i>	<i>Fagus sylvatica</i> y <i>Picea</i> spp.
Kjoller and Clemmensen (2009)	<i>Pezizales</i> sp.	Coniferous
Kjoller and Clemmensen (2009); Qian, Kottke and Oberwinkler (1998)	<i>Piceirhiza nigra</i>	Coniferous
Rineau and Garbaye (2009)	<i>Sebacina epigeia</i> (Berk. & Broome) Neuhoff	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Kjoller and Clemmensen (2009)	<i>Tylospora asterophora</i> (Bonord.) Donk	Coniferous
Kjoller and Clemmensen (2009)	<i>Tomentella</i> sp	Coniferous
Rineau et al. (2010a)	<i>Tomentella sublilacina</i> (Ellis & Holw.) Wakef.	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Rineau et al. (2010a)	<i>Tricholoma</i> sp.	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Kjoller and Clemmensen (2009); Qian, Kottke and Oberwinkler (1998)	<i>Tuber cf. puberulum</i>	Coniferous
Jonsson et al. (1999)	<i>Tylophilos felleus</i> (Bull.: Fr.) Karsten	<i>Picea abies</i>
Qian, Kottke and Oberwinkler (1998)	<i>Tylospora fibrillosa</i> (Burt) Donk,	<i>Picea abies</i>

2.2 Effects of calcareous amendments on the ectomycorrhization of trees

The application of calcareous amendments in natural populations of *Pinus sylvestris*, *Picea abies*, *Quercus petraea* (Mattuschka) Liebl. and *Q. robur* L. have triggered an increase in the number of roots colonised by ECMs; this appears to be related to an increase in soil calcium carbonate and pH (Erland and

Söderström, 1990; Nowotny et al., 1998; Bakker et al., 2000; Borja and Nilsen, 2009). With regard to this effect, Andersson et al. (1996) observed that increased colonisation of ECMs in the host tree roots brings a corresponding increase in the roots' absorption of Ca. In contrast, calcareous amendments have a negative effect on ECM biodiversity. The main ECMs affected by calcareous amendments are acidophilic species which are displaced due to the disappearance of their

ecological niches caused by the increase in soil Ca and pH (Erland and Söderström, 1990; Bakker *et al.*, 2000; Borja and Nilsen, 2009; Rineau and Garbaye, 2009). This modification of the ECM community may be harmful to species of ECM fungi under threat of extinction (Kjoller and Clemmensen, 2009). Tables 2, 3 and 4 compare the ECM species that have been affected by calcareous amendments, positively or negatively, compared to the species of ECMs that were shown to be unaffected.

The amendments appear to have a different effect on the different ECM morphotypes present in the host tree roots. Mycorrhizas without cystidia (smooth morphotypes) such as *Lactarius* spp. and *Russula* spp. decrease their abundance, whereas calcareous

amendments appear to have a positive effect on mycorrhizas with cystidia (rough morphotypes), which spread their colonisation to new roots (Bakker *et al.*, 2000). However, Rineau *et al.* (2010a) observed an increase in the ECMs of *Lactarius subdulcis* in plots of *Fagus sylvatica* and *Picea* spp. (Table 2).

The impact of calcareous amendments on ectomycorrhized roots could be due to the fact that increasing doses of calcium carbonate promote meristematic root cell activity and increase the length of the fine roots. This process appears to be related with an increase in drought resistance observed in various trees in plots treated with calcareous amendments (Qian *et al.*, 1998; Bakker *et al.*, 2000; Rineau *et al.*, 2010a).

Table 3. ECM species whose natural populations decrease in response to calcareous amendments

Authors	ECM species	Host species
Rineau <i>et al.</i> (2010a)	<i>Cenococcum geophilum</i> Fr.	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Rineau y Garbaye (2009)	<i>Clavulina cristata</i>	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Rineau <i>et al.</i> (2010a)	<i>Cortinarius</i> spp	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Rineau <i>et al.</i> (2010a)	<i>Dermocybe</i> spp.	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Kjoller y Clemmensen (2009); Rineau <i>et al.</i> (2010a)	<i>Lactarius</i> spp <i>Lactarius necátor</i> (Bull.) Pers	Coniferous <i>Picea abies</i>
Rineau y Garbaye (2009)	<i>Lactarius tabidus</i> Fr.	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Rineau <i>et al.</i> (2010a)	<i>Paxillus</i> spp.	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Rineau <i>et al.</i> (2010a)	<i>Piceirhiza</i> spp.	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Kjoller y Clemmensen (2009); Rineau <i>et al.</i> (2010a)	<i>Russula</i> sp.	Coniferous
Rineau <i>et al.</i> (2010a)	<i>Russula nigricans</i>	<i>Fagus sylvatica</i> y <i>Picea</i> sp.
Jonsson <i>et al.</i> (1999); Rineau <i>et al.</i> (2010a)	<i>Russula ochroleuca</i> <i>Thelephora terrestres</i>	<i>Picea abies</i>
Jonsson <i>et al.</i> (1999)	Ehrh <i>Xerocomus pruinatus</i>	<i>Picea abies</i>
Rineau y Garbaye (2009)	(Fr.) Quel.	<i>Fagus sylvatica</i> y <i>Picea</i> sp.

Table 4. ECM species whose natural populations do not change in response to calcareous amendments

Authors	ECM species	Host species
Jonsson <i>et al.</i> (1999)	<i>Boletus. Chrysenteron</i> Bull.	<i>Picea abies</i>
Rineau <i>et al.</i> (2010a)	<i>Cantharella sp.</i> <i>Cortinarius anomalus</i> (Fr.: Fr.)	<i>Fagus sylvatica</i> y <i>Picea sp.</i>
Rineau <i>et al.</i> (2010a)	Fr.	<i>Fagus sylvatica</i> y <i>Picea sp.</i>
Kjoller y Clemmensen (2009)	<i>Cortinarius spp.</i> <i>Hygrophorus olivaceoalbus</i> (Fr.)	Coniferous
Rineau <i>et al.</i> (2010a)	Fr. <i>Laccaria amethystina</i> (Bull.)	<i>Fagus sylvatica</i> y <i>Picea sp.</i>
Rineau <i>et al.</i> (2010a)	Murr.	<i>Fagus sylvatica</i> y <i>Picea sp.</i>
Andersson, Jensé y Söderström (1996)	<i>Paxillus involutus</i>	<i>Picea abies</i> y <i>Betula pendula</i>
Kjoller y Clemmensen (2009)	<i>Piceirhiza nigra</i>	Coniferous
Qian, Kottke y Oberwinkler (1998)	<i>Russula ochroleuca</i> <i>Sebacina epigaea</i> Berk. & Broome) Neuhoff	<i>Picea abies</i>
Rineau <i>et al.</i> (2010a)		<i>Fagus sylvatica</i> y <i>Picea sp.</i>
Jonsson <i>et al.</i> (1999)	<i>Tylospora fibrillosa</i>	<i>Picea abies</i>

2.3 Other effects of calcareous amendments on ECM fungi

Rineau and Garbaye (2010) and Rineau *et al.* (2010b) report that the ECMs of *Lactarius* spp. have the capacity to segregate significant quantities of laccase and calcium oxalate CaOx. In their view this explains the accumulations of CaOx crystals observed around the root systems of trees mycorrhized with *Lactarius* spp. in plots treated with calcareous amendments.

Rineau and Garbaye (2009) and Rineau *et al.* (2010a) studied the production of fruiting bodies by ECM fungi in plots treated with calcareous amendments, and report that acidophilic ECMs species fruited closer to the stem of the trees where the soil was more acid. These authors propose *Russula ochroleuca* (Pers.) Fr. as a possible marker species of the effect of calcareous amendments due to the easy identification of its ECMs and the appearance of its carpophores around the trunk of its host trees. Elsewhere, Jonsson *et al.* (1999) and Rineau *et al.* (2010a) did not observe any relation between the biomass of the ECM mycelium

and the production of their fruiting bodies; these same authors also report that this lack of correlation hinders the work of ECM sampling.

García-Montero *et al.* (2009) in the field of truffle cultivation. These authors showed that the application of these amendments in plantations of *Tuber melanosporum* Vittad. and *T. brumale* Vittad. associated to *Quercus* spp. caused a rapid and significant rise in truffle production. However, they pointed out the need for exhaustive studies before applying calcareous amendments in truffle production, owing to lack of knowledge on the potential long-term effects on soil properties and ECM communities.

3. Impact of available soil calcium on the biology of ecm fungi

García-Montero *et al.* (2009) observed that accumulations of Ca and finely divided calcium carbonate (active lime) occur in the rhizosphere of trees mycorrhized by several truffle species (*Tuber melanosporum* and *T. brumale*). García-Montero *et*

al. (2009), therefore, that the ECMs of both these species cause a natural process which is equivalent to “calcareous amendments” to favour their mycorrhization and fruiting. In contrast, low levels of exchangeable Ca and/or soil pH cause a slowdown in the development of summer truffles (*T. aestivum* Vittad.) (Wedén *et al.*, 2009). Furthermore, Aponte *et al.* (2010) report that high levels of Ca and soil pH observed in some natural populations of *Quercus suber* L. and *Q. canariensis* Willd. bear a significant relation with low levels of diversity in the ECM species that colonise them. All these findings suggest that soil Ca is associated with the dynamics of the ECM fungal communities.

When Ca is present in low concentrations in the soil, several species of ECM fungi increase the mobilisation, transport and absorption of Ca by the host trees. These observations explain the finding in experimental plots that Ca concentrations are greater in ectomycorrhized trees than in trees without ECM fungi, when Ca soil availability is low. In these experiments, several authors have shown that the rhizosphere of the ectomycorrhized trees reveals (1) the formation of calcium deposits in the roots, (2) an increase in soil pH, and (3) increased calcium in the litterfall deposited on the soil. Furthermore, different species of ECM fungi can accumulate different amounts of Ca in their mycelium, and as the mycelium of the ECM fungi gradually dies, the accumulation of Ca present in its hyphae are mixed with the soil, thereby increasing the amount of available Ca (Andersson *et al.*, 1996; Aroncena and Glowa, 2000; Zambrano *et al.*, 2009).

4. “Rock-eating” processes associated to ecms and their impact on soil ca

Several authors propose the term “rock-eating” to describe the formation of micro-channels (3 to 10 µm

in diameter) made by the ECM hyphae in feldspar and hornblende rocks rich in K, Ca and Mg (Jongmans *et al.*, 1997; Van Breemen *et al.* 2000a; Blum *et al.*, 2002; Hoffland *et al.*, 2003; Van Schöll *et al.*, 2008). Wallander *et al.* (2003) and Smits *et al.* (2012) have demonstrated that the meteorization of apatite increases with the activity of ECM fungi. These authors indicate that these “rock-eating” processes allow ECM fungi to extract Ca and other nutrients from rocks.

This rock-eating process has not been associated with any types of mycorrhizas other than ECMs. Hoffland *et al.* (2003) and Van Schöll *et al.* (2008) report that endomycorrhizal fungi have very little capacity to absorb Ca from rocks. Blum *et al.* (2002) describe natural populations of *Acer* spp. associated to endomycorrhizas that have a low capacity to access soil Ca, even though this tree species is highly sensitive to Ca deficit. Hoffland *et al.* (2003) and Van Schöll *et al.* (2008) also rule out the involvement of the hyphae of arbuscular fungi in rock-eating processes. In this regard, arbuscular mycorrhizal association plays a key role in the sustainability of terrestrial plant ecosystems, in particular those presenting limitations for the establishment and subsequent growth of plants (Borie *et al.*, 2010). However, a number of authors consider it unlikely that ericoid and saprophytic fungi are involved in these rock-eating processes (Jongmans *et al.*, 1997; Hoffland *et al.*, 2003; Van Schöll *et al.*, 2008).

Rock-eating processes of ECM fungi seem to be associated to a deficit of certain nutrients (K, Ca and Mg) in the soil (Van Breemen *et al.*, 2000a, 2000b; Hoffland *et al.*, 2002; Wallander *et al.*, 2006; Van Schöll *et al.*, 2008). These rock-eating processes begin with the appearance of small cracks or etch-pits on the rock surfaces caused by the secretion of acids (succinate, citrate, oxalate, formate and malate) from the hyphae of ECM fungi (Jongmans *et al.*, 1997;

Hoffland *et al.*, 2003). The density of the micro-tunnels observed in feldspar rocks is correlated with the amount of fine roots existing in the rhizosphere (Hoffland *et al.*, 2003). In certain soils, in one year it is estimated that an average of 166 m of micro-tunnels can be formed per dm³ of rock (Van Breemen *et al.*, 2000a). Van Schöll *et al.* (2008) indicate that there is a different concentration of the ECM hyphae in direct contact with the rock depending on its mineralogical composition. There is an increasing abundance of hyphae in rocks with a mineral concentration following the gradient: Na/Ca-feldspar > K-Feldspar > quartz.

With regard to their position in soils, the greatest rock-eating activity and formation of micro-tunnels by the ECMs occurs in the upper part of the E horizons (Hoffland *et al.*, 2002; Hoffland *et al.*, 2003; Van Schöll *et al.*, 2008).

Blum *et al.* (2002) indicate in regard to the soil-ECM-tree system that rock-eating processes explain the significant quantities of Ca observed in the leaves of mixed conifer and broadleaved forests in Ca-poor soils. These authors note that the Ca accumulated in the leaves came from apatite rocks.

5. Calcium oxalate concentration in the rhizosphere and hyphae of ecms

Zambrano *et al.* (2009) observe that various roots colonised by ECMs revealed accumulations of calcium oxalate crystals (CaOx), with a greater abundance (and larger crystals) than in roots without ECMs. These authors also indicate that the amount of Ca²⁺ and oxalic acid available in the soil of the rhizosphere determines the size of the CaOx crystals. A number of authors report that these accumulations of CaOx are concentrated preferentially on the tips of the ectomycorrhizas and on the hyphae of their fungi, in response to the high concentrations of calcium in

the soil and/or to the abundance of rocks with apatite minerals (Aroncena *et al.*, 2001; Wallander *et al.*, 2003; Zambrano *et al.*, 2009; Rineau and Garbaye, 2010; Smits *et al.*, 2012). Specifically, it has been observed that ECM fungi with extramatricial mycelium such as *Piloderma croceum* Erikss. & Hjortst. accumulate a greater amount of CaOx crystals than fungi without extramatricial mycelium such as *Tomentellopsis submollis* Svrcek. (Weigt *et al.*, 2011).

The morphology and distribution of CaOx crystals vary with diverse ECM morphotypes. In the case of *Piloderma* species, differences were found between the quantities of CaOx accumulated in the rhizosphere on plots studied in the field, and the rhizosphere in plants in the laboratory. This observation was related to the higher number of roots counted in the natural environments in the study (Aroncena *et al.*, 2001; Wallander *et al.*, 2003; Tuason and Aroncena, 2009; Zambrano *et al.*, 2009).

There is also a relationship between the formation of CaOx crystals and the abundance of P in the soil; thus when P was found in limiting conditions for the rhizosphere, the abundance of CaOx crystals formed in the ECM hyphae depended on the level of available soil Ca (Mrnka *et al.*, 2009). A significant relation was observed between the relatively high concentrations of Ca (5mM) and low concentrations of P (0.1 mM) in the soil, and the accumulation of high quantities of CaOx in the rhizospheres. However, in soils with high levels of P, the formation of CaOx crystals was abundant in relation to all values of soil Ca (except in cases where there was no Ca) (Wallander, 2000; Tuason and Aroncena, 2009).

6. Effect of ca in the roots of ecm host trees

Ca accumulation has been observed to occur in the root after calcareous amendments, linked to its increasing concentration in the soil, and according to

Andersson *et al.* (1996), the increased calcium in the roots is distributed throughout the whole of the root system, with no differences noted between the fine roots and the rest; whilst Bakker *et al.* (2000) observed a significant effect of calcareous amendments on root development in *Quercus petraea* and *Q. robur*, which depended on the dose, the type and the age of the plants, and the time elapsed between the amendments and the root sampling. Ostonen *et al.* (2009) found that in ECM host trees, the diameter of the rootlets increases in relation with the concentrations of available soil Ca and N.

In a study of the concentration of various elements present in the roots in several forests of *Fagus sylvatica*, *Fraxinus excelsior* L. and *Tilia* sp. in central Europe, Lang and Polle (2011) and Mrnka *et al.* (2009) noted that root biodiversity plays a key role in the concentrations of root Ca, K, P, Mg and Fe. Thus the concentration of Ca found in the rhizosphere of *Fagus sylvatica* was significantly higher in areas where the roots of the tree were growing alongside the roots of other species. Jentschke *et al.* (1998) and Zambrano *et al.* (2009) indicate that the absorption of soil Ca through roots is depressed by the abundance of soil ammonium, K, Mg and Al, and by low pH values.

7. Effect of ca on the leaves and stems of ecm host trees

Weigt *et al.* (2011) observed that in various seedlings ectomycorrhized with *Tomentellopsis submollis* the Ca concentrations in the stem shoots were greater than the shoots in the seedlings ectomycorrhized with *Piloderma croceum*. In contrast, the roots ectomycorrhized with *P. croceum* had a higher amount of Ca than the roots ectomycorrhized with *Tomentellopsis submollis*.

Ba *et al.* (2002) and Mrnka *et al.* (2009) report that ECM fungi increase the content of nutrients (Ca, N, P, K) in the leaves of certain trees. However these

authors also observed a delayed biomass development in these ectomycorrhized trees.

Other authors have noted a positive correlation between levels of available soil Ca (associated to experimental calcareous amendments), and Ca concentrations found in the needles of various *Pinus* spp. and the leaves of *Betula* spp. (Andersson *et al.*, 1996; Casarin *et al.*, 2004; Borja and Nilsen, 2009). However, Zhang and George (2002) report that in various plots of *Picea abies* no correlation was detected between Ca concentrations in their needles and the calcareous doses added into the soil. These authors suggest as an explanation that soil Al acts as an inhibitor of Ca absorption by *Picea abies*. In this regard, Meriño-Gergichevich *et al.* (2010) indicated that, as result of the negative effects of toxic Al, root metabolic processes, such as water and nutrient absorption, are disturbed with a concomitant decrease in Ca uptake.

Blum *et al.* (2002) determined that the amount of Ca measured in the leaves of trees is significantly related to the Ca extracted and mobilised from feldspar rocks by ECM fungi colonising those trees (through rock-eating processes).

These observations vary based on the species of ECM fungi in question. Mrnka *et al.* (2009) observed that the levels of Ca increased significantly in the needles of populations of *Picea abies* that were mycorrhized by *Hebeloma bryogenes* Vesterh. and *Setulipes androsaceus* L.

Other authors confirm that the Ca present in the leaves may trigger changes in available soil Ca content (when the litterfall is incorporated). Thus the soil is modified by the interactions between different tree species and species of ECM fungi, as each species has a different capacity for absorbing and retaining Ca (Blum *et al.*, 2002; Hobbie *et al.*, 2006; Aponte *et al.* 2010).

Specifically, Aponte *et al.* (2010) indicated that certain deciduous species such as *Quercus canariensis*

incorporate more soil Ca in their leaves than other perennial species such as *Q. suber*. The same pattern has been observed by other authors in natural populations of *Betula pendula* Roth. (deciduous species) versus *Picea abies* (perennial species) in plots treated with calcareous amendments (Andersson *et al.*, 1996).

Aponte *et al.* (2010) also explained that the differences observed between Ca accumulated in the leaves of one deciduous tree species compared to the leaves of one perennial tree species could account for the variations in the amount of available soil Ca (associated to the litterfall) at the base of deciduous trees in comparison with the soil Ca at the base of perennial trees. The litterfall from *Quercus canariensis* supplies 81% more Ca to the soil than the litterfall from *Q. suber*. These authors point out that these differences in the Ca concentrations of the litterfall can lead to selective environmental conditions that would modify the composition of ECM fungal communities.

Aponte *et al.* (2010) confirmed the existence of a negative relationship between the taxonomical biodiversity of ECMs and the Ca concentration in the litterfall from their host trees. In contrast, Borja and Nilsen (2009) did not find any significant relation between the amount of nutrients present in the aciculae of *Pinus sylvestris* and the degree of colonisation of their ECM communities.

8. Discussion

The scarcity of knowledge on the interactions between Ca and ECM fungi has led several authors to highlight the need for new studies on (1) the interaction of litterfall with ECM fungi communities (Aroncena and Glowa, 2000), (2) the interaction of soil Ca and ECM communities (García-Montero *et al.*, 2009), (3) the accumulation of CaOx crystals associated to ECMs (Tuason *et al.*, 2009), (4) the interaction of Ca and tree

roots (Lang and Polle, 2011), and (5) the interaction of ECM rock-eating processes in the nutrition of their host trees (Van Schöll *et al.*, 2008).

The present review reveals that the primary effects of calcareous amendments on ECM fungi communities are, in general: (1) a decrease in their biodiversity (particularly in acidophilic fungi), and (2) an increase in the total colonisation of the roots of host trees by the ECM fungi. Calcareous amendments have varying impacts on ECM communities according to the species and morphotypes of the ECMs, but do not generally have any significant effect on the production of fruiting bodies. Similarly, in experimental plots subject to acid rain, the decrease in soil pH was not found to influence the production of ECM fungi (Agerer *et al.*, 1998). However, the increase in pH and Ca associated to the litterfall from the trees causes variations in the dynamics of ECM fungal communities, and in some cases, a decrease in their carpophore production.

Thy hyphae of ECM fungi are capable of counteracting the lack of Ca and other nutrients in the soil through rock-eating processes. Jongmans *et al.* (1997) and Van Breemen *et al.* (2000) report that the rock-eating processes of ECMs could explain the continued productivity of European forestry in areas affected by acid rain in recent decades. These authors therefore consider the application of calcareous amendments to possibly be unnecessary in acid soils in these regions of Europe.

Certain ECM fungi are capable of accumulating Ca (or its salts) in the rhizosphere. However, there is little knowledge of the causes that provoke these natural accumulations of Ca. Tuason and Aroncena (2009) and Smits *et al.* (2012) proposed that these accumulations of Ca in the rhizosphere could be a precautionary mechanism by ECMs when their soil concentrations decrease. However, the rock-eating activity of ECMs would make it unnecessary to deploy mechanisms for the accumulation of Ca in

order to prevent deficiencies (Wallander *et al.*, 2006; Van Breemen *et al.*, 2000a). Casarin *et al.* (2004) suggests that the natural accumulation of Ca in the rhizosphere of *Pinus pinaster* Ait. could be a “barrier-effect” mechanism by the ECMs that would protect the tree by preventing the absorption of Ca by its roots. Whitney and Arnott (1987) also attribute the accumulations of CaOx to a process of protection of the rhizosphere in response to possible attacks by microbes or by soil fauna.

Finally, several authors have ventured new hypotheses in relation to the interactions between Ca, ECM communities, and other groups of organisms present in forest rhizospheres:

Earthworms may produce the same effect as calcareous amendments on forest rhizospheres, as on the one hand (1) they homogenise the soil, thereby increasing its pH (Qian *et al.*, 1998; Rieneau and Garbaye, 2009); and furthermore (2) earthworms cause an increase in soil calcium carbonate from their calciferous glands (which they all possess). These glands produce a net fixation of C in the soil (from fresh organic matter and CO₂) and an increase in soil pH (Canti, 2009; García-Montero *et al.* 2013). These actions by earthworms would have a similar impact to the effects described for calcareous amendments, as they would similarly modify the nutrients of the forest rhizospheres, alter their ECM communities and modify the growth patterns of the roots.

Certain populations of ECM fungi have the capacity to accumulate Ca in the rhizosphere, which could provoke chlorosis in their host trees and favour their mycorrhization according to a feedback model proposed by García-Montero *et al.* (2009).

9. Conclusion

In conclusion, the present work underlines the fact that soil calcium (and its salts: carbonates and

oxalates) have an impact on the interaction between ECM fungi and host trees. It particularly highlights the impact of calcium on (1) growth patterns in roots and (2) ECM population dynamics. Another noteworthy finding is the importance of Ca cycles (1) in relation to its accumulation and mobilisation by leaves and litterfall, and (2) due to the rock-eating processes associated to ECMs. However, there are still many unanswered questions with regard to the cause-effect relationships between the interactions of Ca and the soil-ECM-plant system in Mediterranean and temperate forests. A greater knowledge of these interactions would have positive repercussions for reforestation projects, soil management actions in these forests, commercial production and harvesting of ECM fungi, and the economy of the various rural areas in these regions.

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