Effects of CO₂ and O₃ on the interaction between root of woody plants and ectomycorrhizae

Xiaona WANG^{a,†}, Eugenios AGATHOKLEOUS^b, Laiye QU^c,
Makoto WATANABE^d and Takayoshi KOIKE^{b,†}

^a College of Landscape Architecture and Tourism, Agricultural University of Hebei, Baoding, 071000 China

^b Silviculture and Forest Ecological Studies, Hokkaido University, Sapporo, 060–8589 Japan

^c Research Center for Eco-Environment Sciences, Chinese Academy Sciences, Beijing, 100083 China

^d Institute of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, 183–8509 Japan

Abstract

The increase of atmospheric carbon dioxide (CO₂) and tropospheric ozone (O₃) concentrations has affected forest ecosystems both above and below ground. A high dose of O₃ alters stomatal function and accelerates foliar senescence, which lead to changes in assimilation and water use efficiency. Consequently, biomass production and plant growth are suppressed by elevated levels of O₃. In contrast, elevated CO₂ ameliorates these responses of plants to high O₃ via stomatal closure. The harmful effect of O₃ on above ground is indirectly linked with below-ground. Importantly, the fine root dynamics and the relationship with symbiotic fungi are significantly influenced. However, the effects of such a changing environment on forest rhizosphere are still poorly understood. The aim of this study is to analyze the combination effect of CO₂ and O₃ on tree roots and ectomycorrhizae. In particular, we summarize and discuss 1) the response of ectomycorrhizal symbionts (colonization rate, community composition, richness, and diversity) under these environmental stresses; and 2) the root dynamics under elevated CO₂ and O₃ concentrations, along with current methodologies used for root sampling.

Key words: Elevated CO₂, Rhizosphere, Root turnover, Symbiosis, Tropospheric O₃.

1. Introduction

Forest ecosystems are threatened by a changing environment due to recent emissions of pollutants (Karnosky *et al.* 2003b). Increasing tropospheric carbon dioxide (CO₂) and ground surface ozone (O₃) concentrations, along with high nitrogen (N) deposition, have become the most phytotoxic air pollutants (Cubasch *et al.* 2001; Matyssek *et al.* 2012; Koike *et al.* 2013).

The concentration of atmospheric CO₂ is continually increasing due to intensive deforestation and use of fossil fuels, with peak already rising up to $\approx 400 \mu\text{mol mol}^{-1}$ (Meehl *et al.* 2007). Furthermore, the O₃ level has been sharply increasing and will extend to a larger region in the next decade (Lelieveld and Dentener 2000), especially in East Asia (Akimoto 2003). Numerous studies have attempted to investigate the effect of elevated CO₂ and O₃ on forest ecosystems. As a result, Free Air CO₂ Enrichment (FACE) and Open Top Chamber (OTC) systems have been developed for research throughout the world (Karnosky *et al.* 2003a, b; King *et al.* 2005; Eguchi *et al.* 2005; Norby and Zak 2011; Desotgiu *et al.* 2013; Koike *et al.* 2015).

In order to understand the tree response, numerous studies have evaluated the effects of elevated CO₂ on ecophysiological processes (Noormets *et al.* 2010; Ellsworth *et al.* 2012) and model estimation (Kauwe *et al.* 2013). Tree growth may increase under

elevated CO₂, accompanied by higher water and N use efficiency (Qu *et al.* 2004; Koike *et al.* 2010; 2015; Norby and Zak 2011). The main conclusion about the effects of elevated CO₂ is an enhancement in net photosynthesis and increased biomass production within a short period (Norby and Zak 2011). Moreover, the carbon (C) gain and allocation below ground are also increased. As an indirect result of these changes, the relationship of tree roots and symbionts is altered (Wang *et al.* 2015b).

Ectomycorrhizae (ECM) fungi represent one economically important group of fungi in the rhizosphere. ECMs form symbiotic relationships with fine roots of a plethora of forest tree species, forming a sheath around the root tips (Tedersoo *et al.* 2010). The fungus then forms a “Hartig Net,” meaning that there is an inward growth of hyphae (fungal cell growth form), which penetrates the plant root structure (Smith and Read 2008).

ECM associations in the rhizosphere play an essential role in forest ecosystems (McNear 2013). Most tree species create symbiotic relationships with ECM fungi to survive in diverse harsh conditions through an efficient nutrient uptake by ECM fungi (Nara *et al.* 2003; Qu *et al.* 2010; Chorianopoulou *et al.* 2015). For instance, in nutrient impoverished soils, ECM fungi preferentially provide nutrients, like phosphorus (P), and water to the above-ground parts of the host trees rather than the direct absorption by the root tissues (Wallander 2000; Alves *et al.* 2010; Chorianopoulou *et al.* 2015). Therefore, ECM trees absorb more organic P and N than non-ECM trees, since ECM fungi produce ectoenzymes (van der Heijden and Sanders 2002; Choi *et al.* 2005; White and Hammond 2008). In return, the symbiotic ECM fungi receive C from photosynthetic assimilation (Högberg *et al.* 2001). Apparently, though, this relationship is unstable under

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[†] Corresponding Author: tkoike@for.agr.hokudai.ac.jp

[†] Corresponding Author: miho54@163.com

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Fig. 1. Schematic overview of the important role of ectomycorrhizal symbiosis and root dynamics in forests C cycling. Rectangles or circles indicate C pools, arrows indicate C fluxes (solid and dashed lines indicate increase and decrease in soil carbon, respectively, and round edges lines indicate that the processes do not always simply decrease the C pool in soil) and single text indicates processes (illustrated partly based on the idea of Fransson 2012; Wang 2015).

changing environmental conditions.

Current concentrations of O₃ have significant adverse effects on forests (Ashmore 2005; Yamaguchi *et al.* 2011; Agathokleous *et al.* 2015b). Most experiments of O₃ effects on trees are based on an assessment of above-ground parts, and much less is known about below-ground processes (Andersen 2003). Exposure of trees to elevated O₃ results in modified C allocation to roots, with further disruptions of the root metabolism and activity of rhizosphere organisms (Scagel and Andersen 1997; McNear 2013). Moreover, root production and mortality play an essential role in the process of C cycling. As it has been reported, C sink retention is greatly regulated by root turnover and below-ground respiration (Fukuzawa *et al.* 2012; Lynch *et al.* 2013; Wang *et al.* 2015a). Significantly, ECM colonization usually occurs in fine root tips, which influences root turnover (King *et al.* 2002). Therefore, the fine root dynamics and ECM symbiosis must be taken into account in a changing environment, as shown in Fig. 1.

Our understanding of below-ground responses to elevated O₃ and CO₂ is still scarce due to several drawbacks such as: inaccessibility of root systems (Andersen 2003; Kasurinen *et al.* 2005; Karnosky *et al.* 2005); deep and wide expanding of roots (Iversen 2010); and interaction of roots with soil bacteria and fungi (Rua *et al.* 2013; Kaiser *et al.* 2015). This review aims to synthesize the current studies dealing with the impact of elevated CO₂ or O₃ on

the rhizosphere (McNear 2013), mainly from two aspects: the ECM species colonization and its diversity with host plants; and the root dynamics under changing environmental conditions.

2. Ectomycorrhizal symbiosis under elevated CO₂ and O₃

Nearly 80% of the terrestrial vascular plant species in global forest habitats rely on mutualistic ECM association to fulfill their dietary needs (Smith and Read 2008). Landeweert *et al.* (2001) reported that a major functional assistance of ECM fungi is the contribution to nutrient uptake of tree through mineral weathering and mobilization of nutrients from soil (Read and Perez-Moreno 2003).

Consequently, growth of the host trees highly depends on the fungal partner, especially in harsh conditions. At the same time, the symbiotic relationship of ECM with host trees is also changed under external stressors. For instance, under the stress of elevated CO₂ and O₃, ECM richness and community composition is altered to defend against or adapt to these stresses. As a result, optimal associations are established between ECM fungi and the host trees (Wang *et al.* 2015b). Fig. 2 shows the kinds of changes that occur in this function under changing air environments.

The effectiveness of ECM fungi in improving tree growth varies, and the nutrition uptake by ECM fungi is often species-specific (Bruns *et al.* 2002) or even strain-specific (Agerer 2001).

Fig. 2. Relation between C allocation to below-ground of a host tree and the ectomycorrhizal symbiosis under elevated CO₂ and O₃. Arrow shows the C driver of different factors (Solid and dashed lines denote enhancement or suppression, respectively) (Wang 2015).

The majority of trees form symbioses with a plethora of fungi species while some of the rest favor specificity, which means the ECM fungi interact with only one host plant (Krause and Kothe 2006; Choi 2008). The reason could be the ECM functional diversity. Plants can control ECM colonization by regulating C allocation to short or fine roots (diameter < 2 mm), according to the intensive relationship between the symbionts and host plants (Hoeksema and Kummel 2003). Thus, specific fungi with low carbohydrate requirements are often favored as symbionts in forest ecosystems.

However, few reports exist on the change in ECM function under changing air environments (Agerer *et al.* 2012). As to ECM functional diversity, much more pertinent experiments are urgently needed to understand the further response of ECM symbiosis to such changing environments. To investigate the nutrient allocation and uptake by ECM fungi, isotope labeling has become an appropriate approach to accurately reach the target with the inoculation of specific ECM species (Trudell *et al.* 2004; Hobbie and Högborg 2012; Teramoto *et al.* 2012).

2.1 ECM colonization rate and species diversity under elevated CO₂

As previously mentioned, the symbiotic relationship between ECM fungi and host trees can be altered according to the C gain and allocation from above-ground to below-ground. Usually, these changes occur first in ECM colonization and later in the ECM community structure (Parrent *et al.* 2006). Increased C supply to below-ground under elevated CO₂ enhances root production and accelerates the growth of host trees (Qu *et al.* 2004). Consequently, this results in a higher demand for mineral nutrients and changes in the abundance of the ECM community.

Elevated CO₂ also increases the mass of fungi, the colonization rate, and the quantity of extrametrical hyphae (Klironomos *et al.* 1997; Tingey *et al.* 2000; Langley *et al.* 2003). However, the effects of elevated CO₂ on ECM richness are not conclusive. In a study of *Pinus taeda* forests, despite elevated CO₂ increased the relative proportion of particular ECM taxa colonizing fine roots and increased the prevalence of unique ECM species, it did not alter the richness and diversity of the whole ECM community (Parrent *et al.* 2006). Furthermore, the response of the ECM community to elevated CO₂ varies among tree species. For instance, Lukac *et al.* (2003) investigated three *Populus* species in

a FACE system and found that the ECM colonization increased only in *P. alba* tree species. Meanwhile, site-specific dissimilarities may occur in ECM communities, as reported in the case of individual *P. taeda* plots (Parrent *et al.* 2006), which is likely to affect the ECM response to the elevated CO₂. Such site-specific dissimilarities have been observed in the lava flow at Mt. Oshima-Koma of Hokkaido island, Japan (Akasaka *et al.* 2007; Kayama *et al.* 2015). In a study of Japanese larch (*Larix kaempferi*) seedlings from three microhabitats in three elevation zones, the results showed that the highest ECM colonization rate occurred in the most shaded micro-habitat with *Larix* understory than in the other two habitats (bare-ground and *Salix reinii* patch). A similar trend was also reported for *Salix* sp. at Mt. Fuji of Honshu island, Japan (Nara *et al.* 2003).

Another factor that may affect ECM diversity is the N content in soil. Parrent *et al.* (2006) found that in *P. taeda* forest plots, an increase of net N mineralization rate was negatively correlated with ECM fungi richness. They also reported that ECM diversity was negatively affected by the N content of the soil. On the other hand, high N loading did not significantly affect the ECM diversity of a hybrid larch F₁ (*Larix gmelinii* var. *japonica* × *L. kaempferi*) and its parents in spite of changes in its community composition and structure (Wang 2015).

The extrametrical mycelia (EMM) of ECM account for a large proportion of the microbial diversity and biomass in forest soils (Cairney 2012). Thus, their response to elevated CO₂ affects plant nutrient acquisition and C movement across forests. In order to assess the response of the EMM to elevated CO₂, Godbold *et al.* (2006) used in-growth bags. Another study about the effects of elevated CO₂ and N fertilization on EMM biomass and ECM community structure was conducted in a *P. taeda* forest by Parrent and Vilgalys (2007), who used sand-filled mesh bags buried in the field and found no increase of EMM biomass in elevated CO₂ plots. They also found no changes in EMM richness or diversity, but the relative frequencies of the EMM community were different. Of the six most abundant taxa, three taxa increased in elevated CO₂ plots (*Tylospora* B, *Tylospora* D, and *Russula* B), two taxa did not differ (*Russula* G and *Atheloid* 3), and one taxon declined (*Theleporoid* 1). Additionally, the changes in ECM fungi biomass may not only be attributed to the tradeoff of assimilated C with or without CO₂ treatment, but also to the morpholog-

ical plasticity of ECM short roots (Ostonen *et al.* 2013; Pritchard *et al.* 2014). Therefore, the morphological plasticity of ECM short roots and the order of ECM short root in a branching system are highlighted for future root research.

2.2 Effect of elevated O₃ on ECM symbiosis

The effects of elevated O₃ on trees are negative compared with those of elevated CO₂. It has been reported that elevated O₃ reduces C assimilation and limits the allocation of photosynthates to below-ground (Grantz and Farrar 2000; King *et al.* 2005; Agathokleous *et al.* 2015b). High O₃ levels may weaken the specific rate of inorganic N uptake by roots (Haberer *et al.* 2007) and decrease the standing crop of fine root and sporocarp production of ECM fungi (Kasurinen *et al.* 2005; Andrew and Lilleskov 2009). Therefore, the nutrient uptake under elevated O₃ exposure is not as efficient as under elevated CO₂. However, the uptake of the essential elements was stimulated by an ECM species-specificity of larch seedlings under elevated CO₂ and O₃ treatment (Wang *et al.* 2015b). This finding evidenced the function of specific ECM species.

The effect of elevated O₃ on ECM colonization is not conclusive. Some studies found that elevated O₃ induced limitation of C allocation to roots, which would be expected to decrease ECM colonization and alter species-host compatibility (Smith and Read 2008; Wang *et al.* 2015b). Conversely, some short-term studies reported an enhancement of ECM formation under O₃ exposure (Rantanen *et al.* 1994; Kasurinen *et al.* 1999). Kainulainen *et al.* (2000) also reported a lack of effects of elevated O₃ on ECM colonization: They found that ECM formation with Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* Karst.) seedlings was unaffected by O₃ after a 3-year fumigation.

Additionally, the symbiosis of ECM with tree roots under elevated O₃ varies over time. There are only a few reports on the response of forest trees to long-term O₃ exposure (Karnosky *et al.* 2003a; Matussek *et al.* 2013), and further exploration about this weak point should be considered in future research.

In this aspect, one potential method for long-term research is to identify local native trees that are sensitive to O₃ and show the typical O₃ foliar injury, and then treat them with ethylene di-urea (EDU) for multi-year monitoring (Paoletti *et al.* 2009; Manning *et al.* 2011; Feng *et al.* 2014; Agathokleous *et al.* 2015a; Saitanis *et al.* 2015). EDU is considered a chemical that offers protection against O₃ and can be used to attribute differences, between experimental conditions, to O₃ (Manning *et al.* 2011; Agathokleous *et al.* 2015a).

3. Root dynamics under changing environment

The root is the hidden half of the plant which regulates plant growth. Roots are roughly classified into coarse roots and fine roots, and fine roots are physiologically more active than coarse ones (Eshel and Beeckman 2013).

Fine roots are usually defined as having diameters smaller than 2 mm (Wang *et al.* 2015a; Agathokleous *et al.* 2016). Although fine roots contribute to less than 2% of tree biomass in forest ecosystems (Brunner and Godbold 2007), they comprise 33-67% of the annual net primary productivity (NPP) in forest ecosystems (Gill and Jackson 2000). Moreover, fine roots grow, die, and decompose very rapidly, so large amounts of C and N cycle

through them annually, even though they have small biomass compared to aboveground tissues (Hendrick and Pregitzer 1992; Ruess *et al.* 2003). The releasing C from below-ground accumulates in soil organic matter and may return to the atmosphere. This movement of C from vegetation to soil is called fine root "turnover" (Tierney and Fahey 2002).

However, it is unclear whether this situation will be maintained over time. Under elevated CO₂ and O₃ concentrations, the pattern will change (Karnosky *et al.* 2005; Norby *et al.* 2005; Grantz *et al.* 2006; Norby and Zak 2011; Koike *et al.* 2015). Given that the NPP of below-ground may exceed 50% of the total NPP (Kubiske and Godbold 2001), such a changing environment may lead to unknown and unpredictable implications for the sustainability of ecosystems. The limited understanding of fine root turnover is a key constraint in quantifying terrestrial C cycling, which is vital for predicting the impacts of global environmental changes (Norby and Jackson 2000). As such, estimation of fine root dynamics is another essential process that cannot be ignored in root research.

So far, it is still difficult to forecast the root lifespan of individuals, populations, or ecosystems with the current knowledge. One explanation for this is the wide variability of the root turnover within and among species (Eissenstat and Yanai 1997). With a stable isotopic analysis, a recently developed approach, Gaudinski *et al.* (2001) suggested that the time of turnover of fine root increases with soil depth. Besides the soil depth, the turnover rate of fine roots differs among different root classes. Pregitzer *et al.* (2008) found that live fine roots with a diameter of 0.5 mm or less and the roots near the surface soil had more rapid turnover than the roots with 0.5-2.0 mm diameter and those in subsurface, respectively. Thus, the effects of soil depth and diameter on root turnover are also valuable to root research (Iversen 2010; Maeght *et al.* 2013). Several methods have been developed for root research, but a standard method for assessing this fine root turnover is still missing (Gill and Jackson 2000; Hertel and Leuschner 2002; Tierney and Fahey 2002; Andersson and Majdi 2005; Iversen 2010; Stover *et al.* 2010; Norby and Zak 2011; Yuan and Chen 2012).

Table 1 lists a comparison of the methods currently in use. A relatively accurate result can be obtained when an appropriate method is chosen for a particular research target. For instance, mini-rhizotrons may sample a more dynamic portion of fine root due to non-destructive installation and successive monitoring of root growth. On the other hand, the extraction of roots from soil cores for ¹⁴C analysis may favor coarse roots (diameter > 0.5 cm) with slower turnover (Tierney and Fahey 2002). According to the strong and weak points of each method, it has been widely suggested that multiple assessment techniques be employed as a means of corroborating estimates, or, in the absence of corroboration, to evaluate the shortcomings of each approach (Fahey *et al.* 1999; Lauenroth 2000; Nadelhoffer 2000; Hertel and Leuschner 2002). Innovative technical research for root turnover is highly recommended. If direct assessment of the root system is difficult and unavailable, the modeling of root growth is an alternative option (Fitter and Hay 2001). However, present attempts represent the very beginnings of root research. More efforts are required for further exploration.

Since the dynamics of fine roots are recognized as a key to bal-

Table 1. The comparison of different methods for root dynamics evaluation.

Methods	Advantages	Drawbacks
Sequential soil coring	a) Standing biomass (Vogt <i>et al.</i> 1998) b) Quick sampling (Neill 1992) c) Simplicity and low cost (Vogt and Persson 1991) d) Coarse roots estimation with ¹⁴ C analysis (Tierney and Fahey 2002) e) Reliable in estimating fine root production when combined with mini-rhizotrons (Hertel and Leuschner 2002)	a) Destructive measurement, thus, b) more replications are needed to overcome the large spatial heterogeneity (Hertel and Leuschner 2002) c) Overestimated root longevity (Vogt <i>et al.</i> 1998) d) May underestimate the belowground net primary production and lead to higher errors than the ingrowth core (Chen <i>et al.</i> 2015)
Ingrowth core	a) Standing biomass (Vogt <i>et al.</i> 1998), which is also more sufficiently reflected than by ingrowth meshes (Andreasson <i>et al.</i> 2015) b) Quick sampling (Neill 1992) c) Simplicity and low cost (Vogt and Persson 1991)	a) Difficult to evaluate the root mortality (Majdi <i>et al.</i> 2005) b) Overestimated root longevity (Majdi <i>et al.</i> 2005)
Mini-rhizotron	a) Non-destructive (Majdi 1996; Johnson <i>et al.</i> 2001) b) More accurate evaluation of root longevity compared with "Sequential soil coring" and "Ingrowth core" c) Longevity tends to be shorter than "Sequential soil coring" and "Ingrowth core" (Majdi <i>et al.</i> 2005) d) Samples a more dynamic portion of fine root (Johnson <i>et al.</i> 2001) e) Available for the evaluation of several characteristics: Diameter (Lopez <i>et al.</i> 2001; Pregitzer <i>et al.</i> 2008); Root length (Johnson <i>et al.</i> 2001; Wang <i>et al.</i> 2015a); Branching characteristics (Liedgens and Richner 2001; Majdi <i>et al.</i> 2001); Frequency of mycorrhizal roots (Leppälammı-Kujansuu <i>et al.</i> 2013)	a) Time consuming for manual processing of images (Zeng <i>et al.</i> 2008) b) High financial cost of instrument and analysis software (Withington <i>et al.</i> 2003) c) Tube installation may generate artifacts in subsequent root data and analysis (Johnson <i>et al.</i> 2001) d) Greater a priori expertise and experience are desirable compared with the other methods

ance the nutrient cycling in forest ecosystems and the C sequestration to soil (Norby and Jackson 2000; Matamala *et al.* 2003; Norby *et al.* 2004; Wang *et al.* 2015a), revealing the fine root survivorship under elevated CO₂ and O₃ is crucial.

3.1 Fine root biomass under elevated CO₂

It is generally accepted that, on average, C distribution within tree body is not changed under elevated CO₂, but trees may sometimes allocate more C to roots than to shoots (Poorter and Nagel 2000; Lukac *et al.* 2009; Poorter *et al.* 2012; Nie *et al.* 2013). Such an effect may lead to changes in below-ground functions of forest ecosystems, such as root production, respiration, and longevity (Pritchard *et al.* 2001). Some below-ground patterns that are likely to appear under elevated CO₂ include: a) an increase in C allocation to fine root production and biomass (Nowak *et al.* 2004; Jackson *et al.* 2009; Lukac *et al.* 2009), and b) an increase in soil C inputs due to fine root turnover (Jastrow *et al.* 2005; Lichter *et al.* 2008; Hoosbeek and Scarascia-Mugnozza 2009). In addition, other similar experiments of elevated CO₂ have shown increased soil respiration (King *et al.* 2004; Pregitzer *et al.* 2006).

Root growth is increased under elevated CO₂ across a range of tree species and experimental conditions. For example, Lukac *et al.* (2003) concluded that standing root biomass of three *Populus* species increased by 47-76% after a FACE treatment. In another study, Pregitzer *et al.* (2008) found that C allocation to below-

ground was positively affected by elevated CO₂, and even the NPP of below-ground may not be offset by negative effects of O₃. Furthermore, elevated CO₂ not only increases root biomass but also affects the root diameter and specific root length. Non-destructive measurements show that elevated CO₂ increases fine-root biomass through an increase in the number and length of fine roots (Thomas *et al.* 1999; Johnson *et al.* 2001; Norby and Zak 2011). However, such influence on fine root growth was not consistently positive. In a recent FACE experiment, elevated CO₂ decreased the total length of fine roots in infertile soil, showing that the effects of CO₂ on roots depend on soil fertility (Wang *et al.* 2015a). In addition, using mini-rhizotron tubes, Thomas *et al.* (1999) found that CO₂ effects on fine root growth was delayed until the second growing season. Therefore, measurements of root growth usually start at least a year after rhizotrons have been inserted (Wang *et al.* 2015a).

3.2 Fine root biomass under elevated O₃

The number of studies focusing on the effects of elevated O₃ on fine root biomass is limited (Grebenc and Kraigher 2007), so conclusions can hardly be drawn. In fact, most of these experiments were conducted with combined elevated CO₂ and O₃ treatment (Kasurinen *et al.* 1999; Pregitzer *et al.* 2008).

In the experiments of Aspen FACE, annual fine-root production and mortality were positively affected by elevated O₃ (Pregitzer *et*

al. 2008; Pregitzer and Talhelm 2013). Soil respiration was greatest in elevated CO₂ and CO₂+O₃ combined conditions, and it was correlated with an increase in fine root biomass. After 10 years of exposure, these experiments found an increased below-ground C allocation and a stimulated fine root biomass in the condition of combined elevated CO₂ and O₃. However, the effects of the combined elevated CO₂ and O₃ treatment on root biomass have been rarely reported and still not clear enough (Matyssek *et al.* 2013).

4. Interaction of ECM symbiosis and fine root longevity

Fine root dynamics and ECM colonization are both affected by elevated CO₂ and O₃, and their responses influence the C cycling in forest ecosystems. ECM colonization is usually stimulated by elevated CO₂ (Lukac *et al.* 2003), even though it just increases for a short period (Shinano *et al.* 2007). Moreover, fine root longevity is altered under elevated CO₂ and O₃ (Lichter *et al.* 2005; Högborg and Read 2006; Wang *et al.* 2015a). Eissenstat *et al.* (2000) concluded that elevated CO₂ might be associated with longer root lifespan, as a result of decreasing the root N concentration and reducing the root maintenance respiration. Arnone *et al.* (2000) also found that root lifespan is longer under elevated CO₂. Additionally, root diameter may change over time, as found in multi-year experiments with elevated CO₂ treatment (Pritchard *et al.* 2008).

The interaction between ECM symbiosis and longevity of fine roots was gradually revealed. Fine roots with ECM symbiosis can live much longer or may have lower production than non-colonized roots (King *et al.* 2002). Such a phenomenon was also demonstrated by Bidartondo *et al.* (2001), who found that roots (diameter = 0.3–0.6 mm) of *Pinus muricata* had longer root lifespan when they were colonized by ECM.

5. Conclusions

We present a partial picture of the effects of elevated CO₂ and O₃ on the ECM symbiosis and fine root dynamics that mediate parts of the rhizosphere in forest ecosystems. However, the understanding of these interactions is still incomplete due to difficulties in studying below-ground processes. It is clear that elevated CO₂ increases the ECM colonization and fine root biomass, but the influence of O₃ exposure still remains unclear. Yet, the complex interaction between ECM symbiosis and fine root dynamics is difficult to understand. Thus, developing novel methodologies to study rhizosphere ecology is a vital target for facilitating progress in root research. Based on modeling and field observations, an optimal and alternative approach for estimating root dynamics should be developed. To conclude, an appropriate method and proper estimation for root turnover that link above- and below-ground processes are critical to profoundly explore the whole rhizosphere, especially under changing environments.

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