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| Title            | Ectomycorrhizal colonization and growth of the hybrid larch F-1 under elevated CO2 and O-3  |
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| Citation         | Environmental Pollution, 197, 116-126<br>https://doi.org/10.1016/j.envpol.2014.11.031   |
| Issue Date       | 2015-02   |
| Doc URL          | http://hdl.handle.net/2115/58552  |
| Туре             | article (author version)  |
| File Information | 69576(Koike_T).pdf  |



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- 1 Ectomycorrhizal colonization and growth of the hybrid larch F<sub>1</sub> under elevated CO<sub>2</sub> and O<sub>3</sub>
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#### 20 Abstract

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22We studied the species abundance and the amount of ectomycorrhizal fungi colonizing a hybrid larch 23(F<sub>1</sub>) under elevated CO<sub>2</sub> and O<sub>3</sub>. Two-year-old larch seedlings were planted in an Open-Top-24Chamber system under four gas treatments: Control (O<sub>3</sub><6 nmol/mol), O<sub>3</sub> (60 nmol/mol), CO<sub>2</sub> (600 25µmol/mol), and CO<sub>2</sub>+O<sub>3</sub>. After two growing seasons the ectomycorrhiza (ECM) colonization and 26root biomass were found to be increased by elevated CO<sub>2</sub>. Ozone impaired the ECM colonization 27and species richness, and reduced the stem biomass. There was no clear inhibition of photosynthetic 28capacity by  $O_3$ , however. The concentrations of Al, Fe, Mo and P in needles were reduced by  $O_3$ , but 29K and Mg in roots increased. This might explain the differences observed in the ECM colonization 30 rate and diversity. No effects of combined fumigation were found for any measured parameter except the P concentration in needles. The tolerance of  $F_1$  to  $O_3$ , as documented, might be a factor in shifts 3132in the ECM community structure.

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34 Key words: Ectomycorrhiza, Elevated ozone, Elevated CO<sub>2</sub>, Hybrid larch F<sub>1</sub>, Species richness

# 35

# 36 Capsule

Elevated  $CO_2$  moderated the negative effects of  $O_3$  on growth of the hybrid larch  $F_1$ , because element uptake by ectomycorrhiza led to a shift in the ectomycorrhizal community structure.

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#### 40 Introduction

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42 Concentrations of atmospheric  $CO_2$  and tropospheric or ground surface ozone  $(O_3)$  have been 43 increasing sharply since the Industrial Revolution. Both are predicted to continue their increase in 44 the coming decades, because of the continued burning of fossil fuels and deforestation (e.g. Cubasch 45 et al., 2001; Tans, 2008; Koike et al., 2013). This rise in atmospheric  $CO_2$  and tropospheric  $O_3$  may 46 affect the above-ground and below-ground growth and development of trees, and therefore impact 47 the CO<sub>2</sub> sink provided by forest ecosystems (e.g. Larcher, 2003; Qu et al., 2010).

48 The majority of below-ground root systems in boreal forests are colonized symbiotically by 49ectomycorrhizal fungi (ECMF) (Taylor et al., 2000). Larch trees (Larix sp.) are typical species 50colonized by ectomycorrhiza (ECM) (Smith and Read, 1997; Qu et al., 2004); they are widely planted in the northeast of Eurasia (Koike et al., 2000; Qu et al., 2010) and part of Europe (Matyssek 5152and Schulze, 1987) as a dominant tree species for afforestation. A hybrid larch  $F_1$  (*Larix gmelinii* var. 53*japonica*  $\times$  *L. kaempferi*; here after F<sub>1</sub>) has recently been developed as a promising species for 54afforestation; it has much better tolerance to cold climate, to grazing damage by the red-back vole 55(*Clethrionomys rutilus*) and to shoot blight disease, as well as tolerance to strong wind (Ryu et al., 2009). The growth of  $F_1$  is closely related to its invariable association with ECM (Ou et al., 2004), 5657although details of this symbiosis are still limited (Qu et al., 2003; 2010). It is estimated that up to 5830% of total photo-assimilation products can be used in growth and maintenance of ECM (Hampp 59and Nehls, 2001). ECM usually act as an efficient resource supplying the root system of the host, by 60 absorbing water and essential nutrients, particularly phosphorus (P) and sometimes nitrogen (N) (e.g. 61 Quoreshi et al., 2003; Cairney, 2011).

62 Many studies have found that the net primary production and growth of trees are enhanced by 63 elevated CO<sub>2</sub>, in which there is also an increase in carbon allocation to below-ground parts (Nowak et al., 2004; Qu et al., 2004; Choi et al., 2005; McElrone et al., 2005). Choi et al. (2005) reported that 6465symbiosis with ECMF increased the growth of seedlings of the Japanese red pine (Pinus densiflora) 66 under elevated CO<sub>2</sub>, because the photosynthetic activities of the host plants were enhanced by an 67 increase in the root surface area via widely ramified ECM hyphae. These authors also found an 68 improvement in water use efficiency (WUE), and suggested that colonization of pine with ECM 69 leads to greater photosynthate allocation to roots under conditions of elevated CO<sub>2</sub>. Colonization by 70 ECM increased the growth of the Japanese larch (*Larix. kaempferi*) and its hybrid larch  $F_1$  by a 71factor of 1.5-2.0 in nutrient-poor soil in northern Japan and eastern Russia (Qu et al., 2004; 2010).

Buscot et al. (2000) emphasized that a greater species richness in ECM communities improves the cycling of P from heterogeneous sources in forest soil ecosystems. Host spruce, larch and pine trees grow more rapidly when they are infected by multiple ECM species than by a single species (e.g. Quoreshi, 2003; Qu et al., 2004; Choi, 2008). Greater ECM species richness may therefore improve nutrient acquisition from different locations and/or soil substrates (Jonsson et al., 2001; Leake, 2001).

In contrast,  $O_3$  usually has negative effects on tree growth. It impairs the physiological and biochemical processes in leaves, and accelerates leaf senescence (Zhang et al., 2002; Matyssek and Sandermann, 2003; Watanabe et al., 2010a; Agathokleous et al., 2014). These negative effects have consequences below ground (Blum and Tingey, 1977; Agathokleous et al., 2014). Carbon assimilation is reduced by  $O_3$ , limiting the below-ground allocation (Grantz and Farrar, 2000; King et al., 2005) and reducing the standing fine-root mass (Kasurinen et al., 2005). These changes are expected to decrease ECM colonization and affect the species-host compatibility.

85 A study of the silver birch (Betula pendula Roth) in Open-Top-Chambers (OTCs) found that 86 O<sub>3</sub> at double the ambient concentration clearly decreased the proportions of black and liver-brown 87 mycorrhizae after three growing seasons (Kasurinen et al., 2005). Haberer et al. (2007) used 88 radioactive isotopes (delta N-15) to measure N uptake and symbiosis of ECM with adult beech trees 89 (Fagus sylvatica). They found that the number of fine roots – which were all mycorrhizal – 90 increased markedly with long-term  $O_3$  fumigation. Other studies, of a 70-year old mixed spruce-91 beech forest stand, found that the number of vital ECM root tips increased, and the ECMF 92community was significantly different after two-year fumigation with O<sub>3</sub> (Grebenc et al., 2007). This 93 is not always the situation, however. Zeleznik et al. (2007) reported ambiguous results for two-year

old beech seedlings exposed to elevated O<sub>3</sub> for two years; there was very little colonization of
seedlings by mycorrhizae, and the ECM types were lower \*\*\*FEWER SPECIES OR LESS OF
EACH SPECIES OR BOTH? CLARIFY – EDITOR\*\*\* under O<sub>3</sub> fumigation than in control plants.
In particular, the number of vital mycorrhizal root tips was reduced by the O<sub>3</sub> treatment (Zeleznik et
al., 2007).

Some previous research has found that ECM colonization decreases under  $O_3$  (Adams and O'Neill, 1991; Edwards and Kelly, 1992) but other work has found an increase (Wöllmer and Kottke, 1990; Gorissen et al., 1991; Kasurinen et al., 1999). The effects of  $O_3$  on ECM symbiosis are therefore not consistent. In fact the effects of  $O_3$  depend on the age of the material plants and the length of fumigation.

External stressors, such as drought and high  $O_3$  concentrations, tend to regulate stomatal conductance. Water-soluble elements are likely to be selectively absorbed by ECM as a defense against the harmful effects of such stresses (Jourand et al., 2014). According to Marjanović et al. (2005a), aquaporin expression is enhanced in ECM seedlings; this enhancement could be particularly important under conditions of water stress (Marjanović et al., 2005b). It follows that, for defense against  $O_3$  stress, element absorption and uptake ability are likely to be adjusted. They are reduced in fine-roots of the European beech under enhanced  $O_3$  (Haberer et al., 2007).

111 Elevated  $CO_2$  and/or  $O_3$  change the ECM community composition by affecting particular ECM 112species. A study of the silver birch (Betula pendula Roth) in OTCs found that elevated CO2 impaired 113light brown/orange mycorrhizae (Kasurinen et al., 2005). In long-term exposure experiments 114involving  $CO_2$  and  $O_3$ , elevated  $CO_2$  alone induced an increase in the proportion of *Sistotrema* spp. 115in the ECM community colonizing aspen/birch trees (Edwards and Zak, 2011). The responses of 116ECM to a combination of elevated  $CO_2$  and  $O_3$  may not be a simple sum of the effects of each. 117According to Volin et al. (1998) the below-ground responses to  $O_3$  were variable, and elevated  $CO_2$ 118typically mitigated the negative effects of O<sub>3</sub> (e.g. Karnosky et al., 2003; Watanabe et al., 2010b). 119The total extent of mycorrhizal colonization of silver birch clones was stimulated by enhanced  $CO_2$ 120and by elevated  $O_3$  separately, but not when combined (Kasurinen et al., 2005).

121 The effects of a combination of elevated  $CO_2$  and  $O_3$  on ECM species richness are not known 122 in detail (Grebenc et al., 2007; Matyssek et al., 2012). We expect the species richness of ECM to 123 depend on the photosynthetic activity of the host plants. Photosynthesis in  $F_1$  seedlings increased 124 under elevated  $CO_2$ , for a short period, but was reduced under elevated  $O_3$  (Koike et al., 2012). We 125 therefore anticipate that colonization by ECM and the species richness in the roots of  $F_1$  should 126 increase under elevated  $CO_2$  and decrease under elevated  $O_3$ .

127 The present work seeks to answer the following questions concerning the hybrid larch  $F_1$  used 128 for afforestation in the coming decades: (1) which ECM species colonize  $F_1$  under elevated CO<sub>2</sub> 129 and/or O<sub>3</sub>; (2) how do the gas treatments influence the ECM species community structure; and (3) 130 what are the effects of these gases on the growth of the hybrid larch  $F_1$ ?

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# 132 2. Materials and Methods

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# 134 **2.1.** Experimental site and plant materials

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Our study was conducted in the Sapporo Experimental Forest of Hokkaido University in northern Japan (43°07′ N, 141°38′ E, 15 m a.s.l.; the annual mean temperature and precipitation in 2011 were 13.5°C and 1254 mm). Two-year-old seedlings of the hybrid larch  $F_1$  (*Larix gmelinii* var. *japonica* × *L. kaempferi*) were provided by the Hokkaido Research Organization Forestry Research 140 Institute near Sapporo. The height and diameter of these seedlings were determined before planting; 141 the mean height of the seedlings at this time was  $38.6 \pm 0.3$  cm, and the mean diameter was  $5.2 \pm 0.2$ 142 mm.

ECM colonization was also determined before planting (see Table 2). The soil at the study site was well homogenized brown forest soil in which there had not been any previous plantation of tree species. All seedlings were planted in May 2011, and were irrigated periodically with tap water to prevent desiccation. Gas treatments began one month later, after all of the seedlings were established at the site. After two growing seasons they were dug out (in late October 2012).

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#### 149 2.2 CO<sub>2</sub> and O<sub>3</sub> treatment

151We set up the OTC system in the experimental forest site of Hokkaido University. The 16 152chambers (dimensions W x W x H =  $1.2 \times 1.2 \times 1.5$  m; 2.2 m high after Sep. 2012) were made of steel frame, with polyvinyl chloride film (Noh-bi, Sapporo, Japan) having a transmittance of 88 % of full 153sunlight cutting UV-B. We set up four gas treatment regimes: (1) control ( $CO_2$  = about 380 µmol 154 $mol^{-1}$ ; O<sub>3</sub>< 6 nmol mol<sup>-1</sup>), (2) elevated O<sub>3</sub> (60 nmol mol<sup>-1</sup>: 7 hours, 10:00-17:00), (3) elevated CO<sub>2</sub> 155(600  $\mu$ mol mol<sup>-1</sup> during daytime) and (4) combination (elevated O<sub>3</sub>+ elevated CO<sub>2</sub>). Charcoal-156157filtered ambient air was introduced, and CO<sub>2</sub> was supplied from a tank in the elevated CO<sub>2</sub> treatments. \*\*\*WAS CO2 SUPPLIED IN AMBIENT TREATMENTS? CONFUSING ORIGINAL 158WORDING - EDITOR\*\*\* The chamber CO<sub>2</sub> concentration was regulated by a control unit (MC-159160F20/S; Koito Co. Ltd., Japan). For O<sub>3</sub> fumigation, O<sub>3</sub> was generated from ambient air using an 161electrical-discharge O<sub>3</sub> generator (IO-1A5; Nippon Ozone Co., Ltd., Tokyo, Japan). In the growth 162chambers,  $O_3$  was continuously monitored by an ultraviolet (UV) absorption  $O_3$  analyzer (TUV-1631100; Tokyo Industries Inc., Tokyo, Japan). The  $O_3$  concentration was less than 6 nmol mol<sup>-1</sup> in the 164untreated O<sub>3</sub> chambers. We set up four replications of each treatment, and four larch seedlings were 165planted in each chamber (64 seedlings in total). A proportional-integrative-differential (PID) control 166 algorithm was applied so as to maintain the desired concentration of  $O_3$ . The monthly data are shown 167in Table 1.

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# 169 2.3 ECM identification

171After 2 years of funigation, we analyzed ECM on the roots of  $F_1$ . After being dug out, all roots 172were covered by wet paper tissue, stored in plastic bags, and transferred immediately to the 173laboratory where they were kept in a refrigerator at 4°C. In not more than 2 days these harvested 174roots were washed until no large clods remained, and then cleaned gently using a small painting-175brush. A microscope (Olympus szx-ILLK100, Japan) was then used to observe the extent of ECM 176colonization, as had also been done prior to planting. A total of 500 root tips were counted randomly 177for each replicate, following the method of Shinano et al. (2007). Fig. 1 shows the sampling process. 178A classification of morphological types of ECM was estimated, \*\*\*HOW?\*\*\* and final 179identification of ECM was carried out by molecular analysis (Table 2), as follows:

First, we extracted ribosomal DNA (rDNA) from the root tips using a DNeasy<sup>TM</sup> Plant Mini Kit (QIAGEN). We then undertook PCR amplification via the polymerase chain reaction by primer 1F/4, in order to determine the sequences of the ITS-region (Gardeset al., 1993; Bellemain et al., 2010). Sequencing reactions were performed using the BigDye Terminator v3.1/1.1 Cycle Sequencing Kit (Applied Biosystems, USA). ECM sequences were then compared with the GenBank database at the DNA Data Bank of Japan, using the basic local alignment search tool (BLAST) program (<u>http://blast.ddbj.nig.ac.jp/blast/blastn?lang=en</u>). 187 The colonization rate of ECM (*CRE*) was calculated from the following formula:

188  $CREi = ER/(ER+NR) \ge 100(\%),$ 

189 where ER and NR rrespectively denote the number of ECM and non-ECM root tips, and i is the 190 ECM type index (Choi et al., 2005; Shinano et al., 2007).

191 The ECM diversity (H') was expressed as Shannon's diversity index (Keylock, 2005) according to 192 the formula:

193  $H' = -\sum_{i=1}^{S} Pi \log Pi,$ 

where S denotes the total number of types of ECM, and Pi is the proportion of the *i*th ECM type(Pielou, 1966).

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# 197 2.4 Measurement of seedling growth and nutrient concentration198

199 The diameter and height of the seedlings were measured in July and November 2011, and 200 again in September 2012. All seedlings were harvested on 20 October 2012 in order to estimate the 201dry mass of plant organs. At this point the seedlings were separated into needles, branches, stems 202and roots. The plant organs were dried in an oven at 80°C for one week and weighed. Needle and 203root samples were crushed into powder by mills and digested by  $HNO_3$ , HCl and  $H_2O_2$ . An 204inductively coupled plasma-atomic emission spectrometer (ICP-AES, IRIS/IRIS Advantage ICAP, 205Thermo Fisher Scientific Inc., Massachusetts, U.S.A.) was then used to determine the concentration 206of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), iron (Fe), 207manganese (Mn) and molybdenum (Mo). The N concentration was determined by the combustion 208method using an NC analyzer (NC-900, Sumica-Shimadzu, Kyoto, Japan).

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# 210 2.5 Measurement of leaf gas exchange rate

Leaf gas exchange rates of the seedlings were measured on the 21-25<sup>th</sup> September 2011 and 11-21215<sup>th</sup> September 2012 using an infrared gas analyzer system (LI-6400, Li-Cor Inc., Lincoln, NE, 213214USA). Two seedlings in each chamber were randomly selected to undergo measurements of their leaf gas exchange rates (8 measurements per treatment). The measurements were conducted on the 215same seedlings throughout the experiment. The net photosynthetic rate (A) and stomatal diffusive 216conductance to H<sub>2</sub>O ( $G_s$ ) were determined at a leaf temperature of 24 ± 0.1°C, 380 µmol mol<sup>-1</sup> CO<sub>2</sub>, 217relative air humidity  $60 \pm 5\%$  and a photosynthetically active photon flux (PPF) of 1500 µmol m<sup>-2</sup>s<sup>-</sup> 218<sup>1</sup>, following Watanabe et al. (2012). Finally, the value of A and the stomatal conductance at the 219220growth concentration [CO<sub>2</sub>] (denoted  $A_{\text{growth}}$  and  $G_{\text{s}}$ ) were measured.

- 221222 2.6 Statistical analysis
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Statistical analyses were undertaken using *R* and SPSS (version 16.0) software. All data were distributed normally, as verified by the Kolmogorov-Smirnov Test. Two-way analysis of variance (ANOVA) was used to test the independent effects of elevated  $CO_2$  and  $O_3$ , as well as their interaction. Tukey's HSD test was applied to identify significant differences between the four treatments. Distance based redundancy analysis (db-RDA) was performed to determine the varying species abundance of the ECM community across the gas treatment regimes.

- 231 **3. Results**
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### 233 3.1 ECM types colonizing $F_1$

We found six types of ECM colonizing  $F_1$  after the treatments, compared with three types before the CO<sub>2</sub> and/or O<sub>3</sub> treatments. According to mycorrhiza taxonomy, eight ECM types belong to either the class Basidiomycetes (Type A, C, D, F, G and H) or Ascomycetes (Type B, E). Table 2 sets out the morphological specification of each type of ECM and the similarity of matched sequences in their identification.

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#### 241 **3.2** Extent of colonization and diversity of ECM

The ECM colonization extent \*\*\*NOT 'RATE' WHICH REFERS TO A SPEED\*\*\* was significantly increased by elevated  $CO_2$ , but was sharply reduced by  $O_3$  relative to the control (Fig. 2). There was no interactive effect of elevated  $CO_2$  and  $O_3$  on the ECM colonization rate, however. The ECM colony varied in diversity across the four treatments (Fig. 2). ECM diversity was significantly reduced by  $O_3$  exposure, and diversity also decreased under elevated  $CO_2+O_3$  treatment relative to the control, whereas a greater diversit wasy found in the control and elevated  $CO_2$ regimes. There was no significant difference between control and elevated  $CO_2$  (*P*=0.49).

251 3.3 Abundance of ECM by species

The six ECM types were found in differing amounts in the four gas treatments. According to the integrated estimation of ECM colonization and species, the major ECM colonizers of  $F_1$  were types A, C, D and F (Fig. 3b). The ECM abundance under elevated O<sub>3</sub> and the mixed fumigation differed significantly from thre control and elevated CO<sub>2</sub> regimes (along the axis-1 direction, 63.7% of the variance was explained, *P*≤0.01). At elevated CO<sub>2</sub> the ECM abundance was similar to that in the control, based on the details of the four ellipses in Fig. 3a.

The number of colonizing species of ECM in the control was unchanged with elevated  $CO_2$ . In a comparison of the particular types of ECM, the proportion of type D was greater and the proportion of type C was less under elevated  $CO_2$  than in the control (Fig. 4a, b). Under exposure to  $O_3$ , type B failed to colonize  $F_1$  whereas type D colonized to a significantly greater extent, and type A colonized less than in the control (Fig. 4c). Under mixed fumigation in which  $CO_2$  and  $O_3$ , were both elevated, the proportion of type C increased relative to the control, and became the dominant species (Fig. 4d).

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#### 3.4 Growth of seedlings and element concentrations

269Ozone markedly reduced seedling growth by the end of the first growing season (Table 3). The 270height and stem diameter of seedlings were not significantly changed by elevated  $CO_2$  relative to the 271control in 2011 and 2012, but these parameters were significantly reduced by  $O_3$  by the end of the 2722011 growing season. Elevated  $CO_2+O_3$  did not exert any effect on the growth of height and stem 273diameter during the two years of treatment. Neither the diameter nor the height was affected by any 274treatment in 2012. Elevated  $CO_2$  increased the biomass of root, stem and total above-ground 275biomass, and O<sub>3</sub> reduced the biomass of stem and root. The combined elevated CO<sub>2</sub>+O<sub>3</sub> did not give 276rise to differences from the control, and there was no interaction between elevated  $CO_2$  and  $O_3$  for 277any biomass parameter (Table 4). The root/shoot (needle + branch + stem) ratio (R/S) was also 278unaffected in every gas treatment.

No clear differences between gas regimes were found for the concentrations of N, K, Ca and

Mg in needles (Table 5). The concentrations of P, Al, Fe and Mo were significantly reduced by  $O_3$ , and concentrations of P and Mn were clearly increased by elevated  $CO_2$ . An interactive effect of elevated  $CO_2$  and  $O_3$  was found in the P concentration. Under the fumigation with elevated  $CO_2+O_3$ , the P concentration in needles increased by a large amount over the control. In roots, only K and Mg were increased by  $O_3$ , and there was no significant effect of the four treatments on the other elements measured.

#### 287 3.5 Gas exchange rate

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In the elevated CO<sub>2</sub> treatment,  $A_{\text{growth}}$  was significantly enhanced in both 2011 and 2012 (Fig. 5). Under elevated O<sub>3</sub> the values of  $A_{\text{growth}}$  did not differ significantly from the control, but did increase with elevated CO<sub>2</sub> and CO<sub>2</sub>+O<sub>3</sub> fumigation in 2011 and in 2012. No significant influence was exerted upon  $G_s$  by any gas treatment. Under both elevated CO<sub>2</sub>+O<sub>3</sub>,  $G_s$  was similar to its control value, but with a tendency to be less in 2011 (*P*<0.1). Ozone tended to induce an increase in  $G_s$  in the second year (Fig. 5).

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- **4. Discussion**
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Overall, the composition of the ECM community was very different by the end of the treatments. *Inocybelacera* (G) and *Thelephoraceae* spp. (H) had colonized  $F_1$  before the treatments, but *Suillus laricinus* (C) and other ECM species replaced them during the 2 years of fumigation. In all treatments, changes in the observed ECM species may be due in part to ECM succession. Nara et al. (2003, 2006) found a common sequence of succession of symbiotic ECMs; for larches, *Suillus* spp. appeared late in the pattern on soul from well weathered lava flow. A similar pattern has been observed in the Japanese larch in a mature forest (Yamakawa, 2012).

305 In general, carbon allocation to below-ground organs increased with elevated  $CO_2$ , in the 306 Japanese larch (Choi et al., 2005) and also other tree species (Nowak et al., 2004; Jackson et al., 307 2009). Carbon allocation to below-ground parts stimulates symbiosis involving ECM (Lukac et al., 308 2003); thus, a significantly increased total ECM colonization of  $F_1$  was observed under elevated 309  $CO_2$ . The diversity was different from the colonization rate pattern under elevated  $CO_2$ , however. 310 This shows that the ECM composition did not change with an increased total colonization rate. In 311fact, the vital support of photosynthates for ECM survival from the host was reduced under  $O_3$  as a 312result of the limited carbon allocation to below-ground (e.g. Grantz and Farrar, 2000). This slowed 313the rate of colonization. Lower ECM diversity was also found under O<sub>3</sub>; we discuss this point later.

The proportion of *Suillus grevillei* (D) increased sharply, and was slightly larger than that of *S. laricinus* (C) in the elevated  $O_3$  regime relative to the control. This is probably because *S. grevillei* colonizes larch seedlings more rapidly than *S. laricinus*, and larch seedlings have higher shoot biomass when colonized by *S. grevillei* than by *S. laricinus* (Qu et al., 2003). Even with reduced ECM colonization, the proportion of favored species increased within the ECM community under elevated  $O_3$  conditions, exactly as *Suillus* spp. did here.

The lower ECM diversity due to  $O_3$  gives rise to a change in the abundance of ECM. \*\*\*CLARIFY WHETHER 'ABUNDANCE' MEANS NUMBER OF DISTINCT SPECIES OR THE TOTAL AMOUNT OF ECM\*\*\* \*\*\*In particular, temporary partners could not have a symbiotic relationship with  $F_1$ —as the species of *Peziza* spp. (B) did in this work. \*\*\*CONFUSING; A TEMPORARY PARTNER DOES HAVE A SYMBIOTIC RELATIONSHIP DURING THE TIME IT IS A PARTNER\*\*\* Also the individuals of *Laccaria cf. laccata* (F) and 326 Tomentella spp. (A) were present in smaller proportions under elevated O<sub>3</sub>, suggesting that the 327assistance and function of these species to the host  $F_1$  was weak, or that their symbiotic activity was 328lower than that of the other ECM types (Suillus spp.). A study of long-term exposure of aspen-birch 329 to elevated CO<sub>2</sub> and O<sub>3</sub> supports our results (Edwards and Zak, 2011). This study found that 330 Laccaria spp. and Tomentella spp. declined together with decreased cello-bio-hydrolase activity in an elevated O3 regime. The ECM colonization rate and diversity were both reduced by elevated 331332 $CO_2+O_3$  relative to the control, but ECM diversity was significantly higher under this combination 333 than under O<sub>3</sub> alone. Suillus spp. colonized the same proportion of roots as in the control. We conclude that, under elevated  $CO_2+O_3$ , the lower diversity induced by  $O_3$  was compensated for by 334 335the effect of  $CO_2$ .

336 The growth (stem diameter and height) and the ECM abundance of  $F_1$  were not accelerated 337 significantly under elevated CO<sub>2</sub>. Nevertheless the increased biomass of stem and root demonstrate 338 that  $F_1$  benefited from elevated CO<sub>2</sub>. The same result has been observed in seedlings of the Japanese 339 larch (Yazaki et al., 2004);  $O_3$  reduced the stem diameter and height at the end of the first growing 340 season. This is a similar result to Noormets et al. (2001), studying aspen; the growth of two aspen 341clones was reduced by  $O_3$  (particularly the stem diameter). The tendency of  $O_3$  to inhibit growth of 342the stem diameter also occurred with potted  $F_1$  plants (Koike et al., 2012).  $O_3$  did not inhibit the 343 growth of F<sub>1</sub> during the second growing season. It appears that F<sub>1</sub> benefited visibly from uptake of 344nutrient elements by ECM in the second year.

345As a symbiotic partners ECM enhances the host plant capability for nutrient uptake of 346 \*\*\*WHAT?\*\*\* (Buscot et al., 2000). We therefore examined the element composition of above- and 347below-ground parts of  $F_1$  plants. With the greater ECM colonization under elevated  $CO_2$ , we found 348 increased concentrations of P and Mn in needles. This increase might be due to increased uptake as a 349 result of the greater ECM colonization. Phosphorus is an essential macro-element for ATP and 350NADPH, and is related to light reactions in photosynthesis (e.g. Reich et al., 2009). Manganese is 351also important for photosynthesis as a co-factor for photosynthetic oxygen evolution (Raven, 1990; 352Henriques, 2003). The uptake of P and Mn enhanced the growth of  $F_1$  and strengthened the 353 symbiosis by presenting a greater opportunity for ECM colonization (Cairney, 2011).

354The concentrations of K and Mg in the roots of  $F_1$  were increased by  $O_3$ . This change may 355assist in maintaining a stable concentration of Fe in roots, since K is vital to maintenance of the iron 356 balance in roots (e.g. Kraemer, 2004). Fe is also essential in the formation of ECM (e.g. Van Hees et 357 al., 2006). We are led to postulate that beneficial and functional ECM partners were previously selected by host  $F_1$  under elevated  $O_3$ . This may explain why the proportions of distinct ECM types 358in the ECM community were different under elevated O<sub>3</sub> than in the other gas regimes. On the other 359hand, concentrations of elements in needles - such as Fe and Mo - were reduced by O<sub>3</sub>, and this 360 361effect might have inhibited the growth of F<sub>1</sub> seedlings. Similar trends have been found by Norby et 362 al. (1986) for oak and by Alina (2001) for many species. The concentrations of N and K did not 363 change significantly, however. Furthermore, the concentrations of Ca and Mg were unaffected, and 364 remained stable in needles of F<sub>1</sub> under elevated O<sub>3</sub>. These two elements are important: Ca is usually correlated with the activity of various enzymes that regulate photosynthesis, and Mg is essential for 365chlorophyll function (e.g. Liang et al., 2009). This might support our hypothesis, \*\*\*REMIND 366 READERS BRIEFLY BY STATING HYPOTHESIS IN A SHORT PHRASE\*\*\* in that there was 367 little inhibition of photosynthesis. On the other hand, under external stress the allocation of nutrients 368 369within the plant is liable to be altered by various ECM species (Weigt et al., 2011). The stable 370 concentrations of Mg and Ca in needles may be due to positive effects of ECM and the changes in 371species abundance. This could be the cause of the weak inhibition of photosynthesis in the  $O_3$ 372treatment. Since the colonizing mycorrhizal species are regulated by their host plants according to

the efficiency of symbionts, especially when carbon allocation to shoots or fine roots changes under external stress, species with lower carbon requirement and/or which utilize carbon effectively are favored (Hoeksema and Kummel, 2003). Further, *Suillus* spp. reportedly reduces the transfer of large quantities of metals towards the plant-fungus interface without impairing normal nutrient uptake to the host plant (Colpaert et al., 2011). In our experiment, the reduction of Al in needles under O<sub>3</sub> was possibly caused by the change in ECM abundance, protecting  $F_1$  from metal toxicity.

379 The net photosynthetic rate ( $A_{\text{growth}}$ ) at elevated CO<sub>2</sub> and elevated CO<sub>2</sub>+O<sub>3</sub> was markedly 380 higher than in the control. Plant growth and root system are usually enhanced under elevated CO<sub>2</sub>, 381and there is accompanying higher water and nitrogen use efficiency of the plant (e.g. Qu et al., 2004; 382Koike et al., 2010; Norby and Zak, 2011). As a result the biomasses of stem and root were increased 383 by elevated  $CO_2$ . We did not find any significant effects on growth and biomass of  $F_1$  of elevated 384  $CO_2+O_3$ , or any interaction effects of the two gases. The value of  $A_{\text{growth}}$  for seedlings under elevated 385  $CO_2 + O_3$  was higher than that for seedlings under elevated  $O_3$  alone, indicating the positive effects 386 of elevated CO<sub>2</sub>. The impact of O<sub>3</sub> on the growth of  $F_1$  seedlings may be mitigated by elevated CO<sub>2</sub> 387 in association with abundant P uptake by ECM, as reported for beech in central Europe (Matyssek 388 and Sandermann, 2003; Weigt et al., 2012). This could explain why the structure of ECM abundance \*\*\*CLARIFY 'STRUCTURE OF ECM ABUNDANCE – DO YOU MEAN SPECIES IN SAME 389390 PROPORTION, OR SAME AMOUNT OF COLONIZATION, OR BOTH?\*\*\* under elevated 391 $CO_2+O_3$  was similar to that of the control. The value of  $G_s$  was not influenced by any gas treatment, 392 IN THE FIRST YEAR? but it was increased by  $O_3$  in the second growing season. This increased the 393 risk of damage due to O<sub>3</sub>; however, the growth of F<sub>1</sub> was found to be unaffected, and A<sub>growth</sub> was not 394reduced by  $O_3$  relative to the control in the second year. It is possible that the increased  $G_s$  is a 395 driving factor for nutrient uptake by the favoring of particular ECM species under O<sub>3</sub> stress. 396

#### 397 **5.** Conclusion

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399 Elevated CO<sub>2</sub> increased the extent of ECM colonization but not the diversity. The higher value 400of Agrowth for F1 under elevated CO2 led to increased biomass of below-ground parts and stem, 401 increasing the ECM colonization rate. Elevated O<sub>3</sub> impaired the extent of ECM colonization and 402 number of species abundance; the latter was very different. \*\*\*DO NOT USE 'ABUNDANCE' AS 403 SHORT FOR 'SPECIES ABUNDANCE' ANYWHERE!\*\*\* Growth of F1 was reduced by O3, and 404 the biomass was reduced. The higher value of  $G_s$  observed under high  $O_3$  did not cause severe 405inhibition of photosynthesis, however, due to the uptake of vital elements by specific ECMs. The 406 changed concentrations of individual elements in needles and roots is related to the change in ECM 407abundance under elevated  $O_3$ . This may have contributed to the defense capability of  $F_1$  seedlings 408 against O<sub>3</sub> by selecting those ECM species capable of flourishing under enhanced O<sub>3</sub>. This point 409 warrants further study with specific ECM species. Elevated  $CO_2+O_3$  together reduced the extent of 410colonization and diversity but increased Agrowth, leading to no overall effect on growth or biomass; 411 this suggests that elevated  $CO_2$  mitigates the harm done by  $O_3$  to photosynthetic capability. A symbiotic partnership between host F1 seedlings and ECM specialists – such as Suillus spp. – may be 412413essential for the survival of F<sub>1</sub> seedlings. Our present results provide information on ECM symbiosis with F1 seedlings under elevated CO2 and/or O3; these results inform us which ar the best ECM 414415species for field inoculation of F<sub>1</sub> to improve plant survival under harsh conditions.

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#### 417 Acknowledgements

We are grateful to Professor Heljä-Sisko Helmisaari of the University of Helsinki for her critical reading of an early draft, to Mr. Evgenios Agathokleous of the Japan Society for the Promotion of Science (JSPS fellow), and we thank everybody who helped to conduct and maintain this experimental system. The present study was partly sponsored by JSPS (Type B: 23380078, 26292075, Type A: 23255009, Young Scientists B 24710027 and B 24780239, and a post-doctoral fellowship for research abroad).

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- 639



641
642 Fig. 1. The sampling process for a single replicate seedling; three subsamples were
643 selected at random.



 $\begin{array}{c} 644 \\ 645 \end{array}$ 

**Fig. 2.** The ECM colonization rate and diversity on the hybrid larch  $F_1$  with differing gas treatments at the end of the experimental period. The ECM diversity (*H*') is calculated as Shannon's diversity index; each value is the average of four chamber replications. A vertical bar indicates the standard error. Different character symbols denote a significant difference between the four treatments; *P*< 0.05. ANOVA: \*\**P*<0.01, \*\*\**P*<0.001, ns denotes not significant.





Fig. 3. Abundance of infecting ECM species in response to four fumigation treatments. The 657 colonization rate \*\*\*EXTENT?\*\*\* involving different ECM species was estimated by distance-658 based redundancy analysis among the four gas treatments. Distance-based redundancy analysis 659 660 depends on the matrix generated from the ECM species and the 4 treatments, and derives the eigenvalues and their contribution to the squared Bray distance. \*\*\*IF THERE ARE MORE THAN 661 4 ECM SPECIES THEN THIS MATRIX IS NOT SQUARE AND IT MAKES NO SENSE TO 662 REFER TO TIS EIGENVALUES?\*\*\* The results of ANOVA use a permutation test for the cap 663 664 scale under a reduced model (CAP1, CAP2 and CAP3 are shown). Independent variables were identified as factors \*\*\*MEANING UNCLEAR\*\*\* (Treat.) CAP1 means axis-1 information, and 665 CAP 2 likewise means axis-2. They are the proportion of the results explained by the difference 666 between each treatment. From the R result we obtain both the direction of axis-1 (P=0.005) and axis-667 6682 (P=0.005), which differ significantly. This results shows that the abundance of colonized ECM 669 species under elevated  $O_3$  and both gases different widely from the control and elevated  $CO_2$  (axis-1 670 direction). The abundance of ECM species also differs markedly between elevated O<sub>3</sub> and both gases (explained via axi- 2). An asterisk denotes significance: \*\*P < 0.01. 671672



 $\begin{array}{c} 674 \\ 675 \end{array}$ Fig. 4. ECM community: changes of abundance in the four treatments. Each value is the proportion 676of all types of ECM identified colonizing the hybrid larch F<sub>1</sub> under 4 types of fumigation (**a**, **b**, **c**, **d**).

677A: Tomentella sp. B: Peziza sp. C: Suillus laricinus D: Suillus grevillei E: Cadophora finlandica F:

- 678 679 Laccaria cf. Laccata.



 $\begin{array}{c} 682 \\ 683 \end{array}$ 

Fig. 5. The net photosynthetic rate at growing [CO<sub>2</sub>] concentrations (Agrowth), and stomatal conductance  $(G_s)$ . Each value is the mean of four chamber replications, and the vertical bar indicates 684685the standard error. ANOVA: \*P < 0.05, \*\*P < 0.01, no asterisk means not significant. Different 686character symbols denote a significant difference between the four treatments; P < 0.05.

687

Table 1

| 690 | Daily concentration (average/peak) of CO <sub>2</sub> (ppm) and O <sub>3</sub> (ppb) for each treatment. |                  |                  |             |             |             |             |             |             |  |
|-----|--|------------------|------------------|-------------|-------------|-------------|-------------|-------------|-------------|--|
|     | Treatment  | 2011.7           | 2011.8           | 2011.9      | 2012.5      | 2012.6      | 2012.7      | 2012.8      | 2012.9      |  |
|     | Control  | 383.3/418.9      | 375.1/417.8      | 392.7/419.7 | 398.8/419.1 | 390.3/419.9 | 386.4/419.7 | 390.9/419.6 | 385.5/416.7 |  |
|     | $CO_2$   | 573.7/582.1      | 570.0/581.7      | 571.1/581.6 | 639.5/660.7 | 624.5/663.2 | 610.4/622.6 | 609.4/620.7 | 603.1/621.7 |  |
|     | O <sub>3</sub>   | _                | _                | _           | _           | _           | _           | _           | _           |  |
|     | $CO_2 + O_3$   | 594.0/608.4      | 592.5/608.7      | 593.7/606.7 | 624.2/643.9 | 615.8/647.4 | 604.8/619.2 | 603.8/619.7 | 595.4/615.7 |  |
|     | Control  | 9.5/19.7         | 9.0/18.9         | 8.9/47.3    | 27.2/38.5   | 13.0/27.3   | 9.6/20.1    | 9.1/18.9    | 8.1/20.9    |  |
|     | $CO_2$   | _                | _                | _           | _           | _           | _           | _           | _           |  |
|     | O <sub>3</sub>   | 52.3/114.2       | 57.3/125.6       | 51.0/ 97.0  | 80.7/104.2  | 67.7/115.8  | 55.3/88.6   | 27.3/84.8   | 39.7/76.0   |  |
|     | $CO_2 + O_3$   | 48.9/123.5       | 53.1/158.5       | 103.4/163.8 | 61.3/74.8   | 62.6/102.6  | 55.2/78.7   | 45.3/93.2   | 45.7/62.9   |  |
| 601 | Each value is  | the average of t | four chamber rer | lications   |             |             |             |             |             |  |

Each value is the average of four chamber replications.

**694 Table 2** 

| 695 | Morpho-type and genetic identification | of ECM species colonizing | hybrid larch F <sub>1</sub> seedlings | before and after the gas treatments. |
|-----|--|---------------------------|---------------------------------------|--------------------------------------|
|-----|--|---------------------------|---------------------------------------|--------------------------------------|

| Treatment | ECM ID | Color         | Ramification <sup>a</sup> | Tip shape <sup>b</sup> | mantle-surface <sup>c</sup> | Emanating hyphae | Accession<br>number | Query<br>cover ECM Species |
|-----------|--------|---------------|---------------------------|------------------------|-----------------------------|------------------|---------------------|----------------------------|
|           | А      | Brown         | 1                         | b                      | 10                          | -                | AB971275            | 99% Tomentella sp.         |
|           | В      | brown-whitish | 5                         | b                      | 12                          | ++               | AB971274            | 98% Peziza sp.             |
| After     | С      | Dark brown    | 2                         | d                      | 11                          | -                | AB971277            | 98% Suillus laricinus      |
|           | D      | brown-whitish | 4                         | d                      | 13                          | +++              | AB971278            | 100% Suillus grevillei     |
|           | E      | Black-brown   | 5                         | с                      | 11                          | +                | EU557316.1          | 100% Cadophora finlandica  |
|           | F      | brown         | 4                         | a                      | 10                          | ++               | AB971276            | 100% Laccaria cf. laccata  |
|           | D      | Dark brown    | 2                         | d                      | 11                          | -                | AB971277            | 98% Suillus laricinus      |
| Before    | G      | light brown   | 4                         | a                      | 12                          | -                | AB971280            | 99% Inocybelacera          |
|           | Н      | Orange brown  | 5                         | b                      | 10                          | -                | AB971281            | 99% Thelephoraceae sp.     |

Note: A symbol '+' indicates the presence of emanating hyphae; the number of '+' symbols increases with their number. The symbol '-' means no hyphae.

<sup>a</sup>See Agerer, pp. 9i-11i

<sup>b</sup> See Agerer, pp. 12i

<sup>c</sup> See Agerer, pp. 13i

# **Table 3**

# 713 Growth in height and stem diameter of hybrid larch $F_1$ seedlings in two growing seasons.

|           |                               | 2011.07                    |              | 2011.11                     |                              | 2012.09                     |                               |
|-----------|-------------------------------|----------------------------|--------------|-----------------------------|------------------------------|-----------------------------|-------------------------------|
|           |                               | Diameter(mm)               | Height (cm)  | Diameter (mm)               | Height(cm)                   | Diameter (cm)               | Height (cm)                   |
| Treatment | Control                       | 5.51( <mark>0.20</mark> )a | 38.65(1.42)a | 9.60( <mark>0.39</mark> )a  | 72.98( <mark>4.37</mark> )a  | 16.69( <mark>1.13</mark> )a | 202.69(14.02)a                |
|           | CO <sub>2</sub>               | 5.00( <mark>0.21</mark> )a | 38.68(1.72)a | 9.53( <mark>0.52</mark> a   | 73.05( <mark>5.93</mark> )a  | 17.28( <mark>1.29</mark> )a | 198.39( <mark>18.56</mark> )a |
|           | O <sub>3</sub>                | 5.08( <mark>0.12</mark> )a | 38.78(1.19)a | 7.37( <mark>0.36</mark> )b  | 55.74( <mark>3.11</mark> )b  | 14.89( <mark>1.16</mark> )a | 173.44( <mark>14.43</mark> )a |
|           | $CO_2+O_3\\$                  | 5.00( <mark>0.20</mark> )a | 38.24(1.57)a | 8.80( <mark>0.41</mark> )ab | 67.86( <mark>3.63</mark> )ab | 16.54( <mark>0.84</mark> )a | 203.75( <mark>8.84</mark> )a  |
| ANOVA     | CO <sub>2</sub>               | ns                         | ns           | ns                          | ns                           | ns                          | ns                            |
|           | O <sub>3</sub>                | ns                         | ns           | **                          | *                            | ns                          | ns                            |
|           | $\text{CO}_2\times\text{O}_3$ | ns                         | ns           | ns                          | ns                           | ns                          | ns                            |

Each value is the average (SE) of four chamber replications. ANOVA: \*P<0.05, \*\*P<0.01, ns not significant. Different character symbols denotes a

715 significant difference between the four treatments; P < 0.05.

# **Table 4**

718 Dry mass of plant organs and the ratio of root biomass to shoot biomass (R/S) for hybrid larch F<sub>1</sub> seedlings at the end of the experimental period.

|        |  | Needle                      | Branch                      | Stem                         | Root                         | Above ground                  | R/S 719                    |
|--------|--|-----------------------------|-----------------------------|------------------------------|------------------------------|-------------------------------|----------------------------|
| Treat. | Control                                | 37.57( <mark>3.61</mark> )a | 34.91(3.75)a                | 44.66(5.38)ab                | 36.88(4.02)ab                | 116.23(10.97)ab               | 0.32( <mark>0.02</mark> )a |
|        | $CO_2$                                 | 48.22(7.59)a                | 41.12( <mark>8.88</mark> )a | 51.67( <mark>6.50</mark> )a  | 49.29( <mark>6.07</mark> )a  | 144.76( <mark>22.43</mark> )a | 0.36( <mark>0.04</mark> )a |
|        | <b>O</b> <sub>3</sub>                  | 32.92(5.53)a                | 20.82( <mark>3.63</mark> )a | 28.64( <mark>4.87</mark> )b  | 25.57( <mark>4.17</mark> )b  | 082.38(13.70)b                | 0.33( <mark>0.04</mark> )a |
|        | $CO_2 + O_3$                           | 47.07( <mark>6.68</mark> )a | 37.50(5.54)a                | 45.21( <mark>4.29</mark> )ab | 40.13( <mark>3.86</mark> )ab | 129.78(15.08)ab               | 0.32(0.02)a                |
| ANOVA  | CO <sub>2</sub>                        | ns                          | ns                          | *                            | **                           | *                             | ns                         |
|        | <b>O</b> <sub>3</sub>                  | ns                          | ns                          | *                            | *                            | ns                            | ns                         |
|        | $\text{CO}_2 \!\!\times \! \text{O}_3$ | ns                          | ns                          | ns                           | ns                           | ns                            | ns                         |

720 Each value is the average (SE) of four chamber replications. R/S: Ratio of root biomass to shoot biomass (needle+branch+stem). ANOVA:

P<0.05, \*\*P<0.01. Different character symbols denote a significant difference between the four treatments; P<0.05.

# **Table 5**

| Organs | Treatment                       | Nutrient concentration (mg/g) |                            |                             |                            |                            |                            |                            |                            |                            |  |  |
|--------|---------------------------------|-------------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|--|--|
|        |                                 | Ν                             | Р                          | К                           | Ca                         | Mg                         | Al                         | Fe                         | Mn                         | Мо                         |  |  |
| Needle | Control                         | 20.42( <mark>6.16</mark> )a   | 1.62(0.18)b                | 10.21 <mark>(0.63)</mark> a | 7.67 <mark>(1.76</mark> )a | 1.25 <mark>(0.20)</mark> a | 0.12(0.02)ab               | 0.14(0.02)a                | 0.04( <mark>0.03</mark> )a | 0.03(.002)a                |  |  |
|        | $CO_2$                          | 19.10( <mark>6.37</mark> )a   | 2.0 <mark>9</mark> (0.26)b | 11.44( <b>1.03</b> )a       | 5.20( <mark>0.78</mark> )a | 0.96( <mark>0.12</mark> )a | 0.12( <mark>0.01</mark> )a | 0.13( <mark>0.03</mark> )a | 0.09( <mark>0.06</mark> )a | 0.03(.004)a                |  |  |
|        | <b>O</b> <sub>3</sub>           | 18.43( <mark>6.14</mark> )a   | 1.91(0.18)b                | 6.95( <mark>0.77</mark> )a  | 4.61( <mark>0.64</mark> )a | 0.98( <mark>0.22</mark> )a | 0.07( <mark>0.02</mark> )b | 0.09(0.02)a                | 0.04( <mark>0.01</mark> )a | 0.01(.002)b                |  |  |
|        | CO <sub>2</sub> +O <sub>3</sub> | 18.54(5.14)a                  | 4.13(0.51)a                | 8.83(2.25)a                 | 4.44( <mark>0.59</mark> )a | 0.88( <mark>0.09</mark> )a | 0.07(0.01)b                | 0.09(0.02)a                | 0.08( <mark>0.02</mark> )a | 0.01(.002)b                |  |  |
| ANOVA  | $CO_2$                          | ns                            | **                         | ns                          | ns                         | ns                         | ns                         | ns                         | **                         | ns                         |  |  |
|        | O <sub>3</sub>                  | ns                            | **                         | ns                          | ns                         | ns                         | **                         | *                          | ns                         | ***                        |  |  |
|        | $CO_2 \!\!\times\!\! O_3$       | ns                            | *                          | ns                          | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         |  |  |
| Root   | Control                         | 16.37( <mark>4.09</mark> )a   | 2.47( <mark>0.11</mark> )a | 3.90( <mark>0.35</mark> )a  | 9.38( <mark>0.44</mark> )a | 2.07( <mark>0.06</mark> )a | 6.10( <mark>0.30</mark> )a | 4.19( <mark>0.06</mark> )a | 0.07(0.01)a                | 0.01(.001)a                |  |  |
|        | $CO_2$                          | 15.74 <mark>(4.37)</mark> a   | 2.13( <mark>0.17</mark> )a | 4.15( <mark>0.23</mark> )a  | 8.75( <mark>0.74</mark> )a | 1.96(0.15)a                | 5.86 <mark>(0.70</mark> )a | 4.02( <mark>0.24</mark> )a | 0.09(0.01)a                | 0.01 <mark>(.003)</mark> a |  |  |
|        | <b>O</b> <sub>3</sub>           | 15.93 <mark>(3.98)</mark> a   | 2.30( <mark>0.29</mark> )a | 5.09( <mark>0.62</mark> )a  | 7.76( <mark>2.00</mark> )a | 2.40( <mark>0.17</mark> )a | 6.48( <mark>0.52</mark> )a | 4.39( <mark>0.13</mark> )a | 0.09(0.01)a                | 0.01(.001)a                |  |  |
|        | $CO_2 + O_3$                    | 17.22( <mark>4.30</mark> )a   | 2.58( <mark>0.24</mark> )a | 4.75( <mark>0.29</mark> )a  | 8.97( <mark>0.58</mark> )a | 2.30( <mark>0.17</mark> )a | 6.32( <mark>0.70</mark> )a | 4.37( <mark>0.08</mark> )a | 0.10(0.01)a                | 0.02(. <mark>000</mark> )a |  |  |
| ANOVA  | $CO_2$                          | ns                            | ns                         | ns                          | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         |  |  |
|        | O <sub>3</sub>                  | ns                            | ns                         | *                           | ns                         | *                          | ns                         | ns                         | ns                         | ns                         |  |  |
|        | $CO_2 \times O_3$               | ns                            | ns                         | ns                          | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         |  |  |

| 724 | Effect of elevated $CO_2$ and | d O <sub>3</sub> on nutrient | concentration in | needles and | l roots of seedlings. |
|-----|-------------------------------|------------------------------|------------------|-------------|-----------------------|
|-----|-------------------------------|------------------------------|------------------|-------------|-----------------------|

Each value is the average (SE) of four chamber replications. ANOVA: \*P < 0.05, \*\*P < 0.01, \*\*P < 0.001, ns not significant. Different character symbols

726 denotes a significant difference between the four treatments; P < 0.05.

# 728 Appendix











Note: Each photo shows typical morphology of the different ECM species; black bar=1
mm.

- A: Tomentella sp., B: Peziza sp., C: Suillus laricinus, D: Suillus grevillei, E: Cadophora
- 737 *finlandica*, F: *Laccaria* cf. *laccata*, G: *Inocybelacera*, H: *Thelephoraceae* sp.