Elevated in-soil CO$_2$ affects physiology and growth of *Pinus densiflora* and *Quercus variabilis* seedlings under an artificial CO$_2$ release experiment

Hyun-Jun KIM$^{1,2}$, Seung Hyun HAN$^{3}$, Seongjun KIM$^{1,2}$, Hanna CHANG$^{1}$, Yowhan SON$^{1,4}$

$^1$Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Republic of Korea
$^2$Department of Forest Resources, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, Republic of Korea
$^3$Restoration Center for Endangered Species, National Institute of Ecology, Yeongyang, Republic of Korea

**Abstract:** It is important to understand how woody species are affected by elevated in-soil CO$_2$ for carbon capture and storage (CCS). A study was conducted to analyze the effects of artificially released in-soil CO$_2$ on the physiology and growth of 4-year-old *Pinus densiflora* and 3-year-old *Quercus variabilis* seedlings. Approximately 7.9 kg CO$_2$ plot$^{-1}$ d$^{-1}$ was released at a depth of 0.5 m over the period from 20 June to 20 July 2017. For both species, chlorophyll fluorescence and content, photosynthetic rate, and leaf size significantly decreased after the CO$_2$ release. However, stomatal behavior varied between these species under the elevated in-soil CO$_2$ conditions. Elevated in-soil CO$_2$ inhibited plant physiological functions by limiting available in-soil O$_2$. The leaf size of treatment plots showed significantly lower values of 0.60 ± 0.05 cm$^2$ for *P. densiflora* and 12.05 ± 1.47 cm$^2$ for *Q. variabilis* compared to those of control plots of 0.90 ± 0.09 cm$^2$ for *P. densiflora* and 21.84 ± 3.62 cm$^2$ for *Q. variabilis*, whereas the number of leaves increased from 2697 ± 153 leaves to 3121 ± 255 leaves for *P. densiflora* and from 95 ± 4 leaves to 288 ± 52 leaves for *Q. variabilis*. It was found that the decrease in leaf size resulted in a second flush, which increased the total leaf area per seedling. The biomass of *P. densiflora* significantly decreased in the treatment plots (P < 0.05). *Q. variabilis* showed an increase in mortality, with a low percentage of fine root (<2 mm in diameter) with respect to the total root biomass (P < 0.05). These results indicated that the physiological responses to elevated in-soil CO$_2$ are more sensitive than the growth responses for both species.

**Key words:** Carbon allocation, carbon capture and storage (CCS), elevated in-soil CO$_2$, leakage, O$_2$ depletion, second flush

1. Introduction

The increase in atmospheric CO$_2$ affecting the climate and environment has become an issue of international interest (Hansen, 2004; Norby and Luo, 2006). In an IPCC special report (IPCC, 2005), carbon dioxide capture and storage (CCS) was introduced as an effective technology to reduce the effects of climate change. CCS is a component of solution portfolios with the potential to reduce CO$_2$ emissions. The process of CCS involves the capture from major industrial sources of CO$_2$ that would otherwise be emitted to the atmosphere and stored underground (Bachu, 2000). However, even when CO$_2$ is successfully captured, there are a number of events that might incur CO$_2$ leakage, such as injection and abandoned wells, fault surfaces, hydraulic fractures, and earthquakes (Hepple, 2002; Wilson et al., 2003; Benson et al., 2005). The release of a high concentration of CO$_2$ in a relatively short period of time could negatively affect not only human society but also terrestrial and aquatic ecosystems (Price et al., 2007; Kim et al., 2018b).

To ensure the safe application of CCS technology, it is important to analyze the effects of CO$_2$ leakage on these ecosystems (Kim et al., 2017a). In this study, plants were used as a simple and cost-effective bioindicator of CO$_2$ leakage from geological formations, such as oil and gas fields, unmineable coal beds, and deep saline formations (IPCC, 2005; Stalker et al., 2012; Patil, 2012). According to previous studies that conducted environmental risk assessments of CO$_2$ leakage on herbaceous plants (wheat and grass; Pierce and Sjögersten, 2009; Feitz et al., 2014), and crops (corn and Chinese cabbage; Wu et al., 2014; Kim et al., 2017b, 2017c), the increase in in-soil CO$_2$ reduced the growth rates of the study plants, although the tolerance to elevated CO$_2$ varied depending on the species (Lichtenthaler, 1996; Noomen and Skidmore, 2009).

However, previous studies focused on herbaceous and crop plants because most of the CCS commercial sites are located in grasslands. For other sites having large forest area and small grasslands, studying the responses of woody plants to elevated in-soil CO$_2$ is also necessary, but studies...
performed on woody plants are rare. As perennial woody plants can be monitored long term due to their relatively long growth period compared to that of herbaceous plants and crops, they are suitable for the long-term monitoring of CO2 leakage. However, there is a lack of research on the effects of a high level of in-soil CO2—either natural or anthropogenic—on woody plants (Pierce and Sjögrensten, 2009).

To evaluate how woody plants respond to elevated in-soil CO2, we investigated Pinus densiflora and Quercus variabilis seedlings, which are common plantation species and are important from an ecological and socioeconomic point of view (Byun et al., 2010). It is hypothesized that: (1) increased in-soil CO2 might inhibit physiological function and growth of study species by reducing in-soil O2; however, (2) plants’ responses can differ depending on their species-specific physiological traits. (3) While some species show a tendency to prioritize carbon uptake over water loss by retaining their stomata even under condition of water stress, others prioritize preventing dehydration by closing stomata at the expense of reduced carbon uptake. (4) Therefore, the response of plants to increased CO2 may vary depending on the species.

2. Materials and methods

2.1. Experimental CO2 release site and design

The study site, named the Environmental Impact Evaluation Test Facility on the Seepage of Geologically Stored CO2 (EIT), was established at Eumseong-Gun, Chungcheongbuk-Do, South Korea (36°57′44.2″N, 127°28′03.1″E) in 2015. This study site comprises a saturated zone, an unsaturated zone, and a control facility (Kim et al., 2018b). The saturated zone consists of soil layers without pores and which are under water, and the unsaturated zone consists of soil layers between the surface and the water table. In other words, the water table acts as a boundary between the saturated and unsaturated zones. An environmental risk assessment of soil and plant ecosystems in the unsaturated zone, which was further divided into 5 zones, was conducted. Zone 1 consisted of woody plants. Zones 2 and 4 were cropland. Zone 3 was a nonplantation area, and Zone 5 contained both woody plant and crops. This study was primarily concerned with the effects of elevated in-soil CO2 on the plants in Zone 1.

Over the previous 5 years, the mean annual temperature and mean annual precipitation at the study site had been 11.7 °C and 1315.6 mm, respectively (Kim et al., 2018a). Sandy loam soil was observed down to 1 m depth, and about 30 m of weathered soil was located under the sandy loam soil (Kim et al., 2018b).

Zone 1 consisted of 4 compartments, arranged in a 2 x 2 format (Figure 1). Two of the compartments were designated for pines and the other two for oaks. Neighboring compartments were designed to be different for specific species in order to minimize the influence of compartment position on seedling growth. Each compartment comprises 2 control and 2 CO2-enriched plots (4 m x 2 m each). Two-year-old P. densiflora and 1-year-old Q. variabilis seedlings were planted 20 cm apart (a total of 200 seedlings per plot) in May 2015 (Figure 1a). Among these seedlings, only 20 seedlings within a 1 m x 0.8 m area from the center of each plot were used to analyze the physiological and growth responses of P. densiflora and Q. variabilis seedlings to elevated in-soil CO2 (Figure 1b).

To enrich CO2, a gas-sensor probe tip (Retract-A-Tip, AMS Inc., American Falls, ID, USA) was inserted to a soil depth of 0.5 m (at an angle of 45°; Figure 1c), and approximately 7.9 kg CO2 plot−1 d−1 (total 1896 kg CO2) was instilled at a rate of 3 L min−1 during the period 20 June–20 July 2017. The Artificial Soil Gassing and Response Detection (ASGARD) experiment in UK was referred to for the CO2 release rate. A regulator was used to control the constant CO2 injection rate. The Retract-A-Tip was removed after each use for subsequent use rather than leaving it inserted. One CO2 generator per plot was installed in the middle of each plot (Figure 1a).

2.2. Monitoring of environmental factors

In-soil CO2 and O2 concentrations were measured at 5 points in each plot using a GA5000 (Geotech, Coventry, UK) on 14 and 21 June, 5 and 19 July, and 2 August 2017 (Figure 1b). Special PVC pipes for in-soil CO2 measurements were placed at all CO2 concentration measuring points at a depth of 0.15 m. The GA5000 was designed to measure in-soil gas with an accuracy of ± 0.5% CO2 and ± 1.0% O2 (Operating Manual). CO2 efflux was also measured using a GMP343 probe (Vaisala, Finland) at the same points and on the same dates as the CO2 concentration measurements. The GMP343 probe was designed to give a highly accurate CO2 measurement, which was ±0.5 ppm CO2 at 25 °C and 1013 hPa (User’s Guide), and was based on the nondispersive infrared (NDIR) sensor (Kim et al., 2018b). The change in CO2 concentration over 5 min was evaluated by measuring the CO2 concentration every 15 s using a cylindrical chamber having a diameter of 119 mm and a length of 155 mm. CO2 efflux was estimated using the following equation (Kim et al., 2018b).

\[
F_{CO2} = (P + V) / 1000 \times (273.15 + 10.000) / (A \times (T + 273.15)) \times ΔCO2 / Δt \times 44 / 22.41
\]

Here, \(F_{CO2}\) is the CO2 efflux (μg CO2 m−2 s−1), P is atmospheric pressure (kPa), V is the volume of the chamber (cm3), A is the basal area of the chamber (cm2), T is gas temperature (K), ΔCO2/Δt is the initial rate of change in CO2 mole fraction (µmol mol−1), and 44/22.41 is the weight of a CO2 molecule (at 1 mol of atmospheric volume [g mol−1]).

2.3. Physiological responses

In general, solar energy consists of photosynthesis, fluorescence, and heat. Photochemistry is the energy that plants absorb through photosynthetic processes, such as photosynthesis. Fluorescence and heat are the energy that plants release through nonphotochemical processes. When plants are under stress, chlorophyll fluorescence increases. For this reason, chlorophyll fluorescence is used as an index of stress experienced by plants. For chlorophyll fluorescence analysis, 3 sample seedlings per plot were randomly selected. After the sample leaves were dark-adapted using a small and lightweight leafclip for 1 h, fluorescence origin (Fo), fluorescence maximum (Fm), variable fluorescence (Fv), and photosynthesis efficiency (Fv/Fm) were measured 3 times at a light intensity of 1500 μmol m−2 s−1 using a Handy PEA (Hansatech Instruments Ltd., Norfolk, UK) on 14 and 21 June, 5 and 19 July, and 2 August 2017. Fo is a parameter thought to represent emission by excited chlorophyll a molecules in the antennae structure of Photosystem II. Fm is termed maximum fluorescence if the light intensity is fully saturating for the plant and the electron acceptor Qa is fully reduced. Fv indicates the variable component of the recording and relates to the maximum capacity for photochemical quenching. Fv/Fm is a parameter widely used to indicate the maximum quantum efficiency of Photosystem II.

Figure 1. (a) Location of plots and CO2 injection pipeline, (b) positions of seedlings, CO2 efflux, concentration measuring points, CO2 release point, and soil temperature and moisture measuring point in each plot, and (c) profile of a CO2 injection system for the artificial CO2 release experiment.
The amount of chlorophyll in the leaves was analyzed on 16 June, 21 July, and 18 August 2017 using the dimethyl sulfoxide (DMSO) extraction method developed by Hiscox and Israelstam (1979). Two leaves were randomly taken from 3 sample trees from each plot; for P. densiflora seedlings, 2 needles surrounded by a sheath were treated as one leaf sample. The collected leaves were cut to a length of 1 mm, 0.02 g of fresh leaves were placed in a vial bottle, and 5 mL of DMSO was added. The absorbance of the liquid samples was measured using a spectrophotometer (UH53, Hitachi, Tokyo, Japan) after the vial bottles were soaked in a water bath at 65 °C for 6 h to extract the chlorophyll. At this time, the blank was measured using only the DMSO method, and the absorbance was set to zero. Finally, the chlorophyll content was measured using the method explained by Barnes et al. (1992) as follows:

Total chlorophyll (μg mg⁻¹) = (20.34 × A648 + 7.49 × A665) × V / FW.

Here, A648 and A665 are absorbance values measured at 648 nm and 665 nm wavelength, respectively, V is the DMSO dose, and FW is the fresh leaf weight (mg).

The net photosynthetic rate (Fp), stomatal conductance (Gs), and transpiration rate (E) were determined 3 times for each measurement using a portable photosynthetic analyzer (CIRAS-2, PP-Systems, Amesbury, MA, USA) over the period from 15 June to 3 August 2017 after 3 sample trees per plot were randomly selected. These were measured between 08:00 and 12:00 because they were affected by atmospheric temperature (Kirschbaum, 2004). The light intensity was maintained at 1200 μmol m⁻² s⁻¹ using an LED light module, and the CO₂ concentration was controlled at 400 ppm. Air humidity and the leaf surface temperature were set as ambient and 25 °C, respectively. The number, width, length, and area of the leaves were measured using a scanner (Perfection V700, Epson, Tokyo, Japan) and leaf area analysis program (WinSEEDLE, Regent Instruments, Québec, Canada). The total dry weight (TDW), number of leaves (NL), mean leaf area (MLA), and total leaf area (TLA) were measured on 18 August 2017.

2.4. Growth responses

Three seedlings among 20 sample seedlings per plot were gathered on 18 August, separated into roots, stems + branches, and leaves in the laboratory, and dried in a dryer (OF-22 GW, Jeio Tech Co. Ltd., Seoul, South Korea) at 65 °C for 72 h. The roots were classified into coarse root (≥ 2 mm) and fine root (<2 mm) based on their diameter to obtain the percentage of fine root with respect to the total root biomass. The dry weight of all tissues was calculated. Mortality was estimated by counting the number of living seedlings before gathering the biomass samples on 18 August 2017.

2.5. Statistical analysis

The differences in Fv/Fm, chlorophyll content, Pn, Gs, E, and biomass between the plants in the control and CO₂-released treatment plots were analyzed using one-way ANOVA. Duncan’s multiple range test was performed to determine the significant difference between the two groups at a significance level of α = 0.05. T-test analysis was used to analyze the differences in TDW, NL, MLA, and TLA between the control and treatment plots at a significance level of α = 0.05. The coarse and fine root contents were also analyzed by using T-test analysis. SAS 9.4 software (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses conducted in this study.

3. Results

3.1. Monitoring of in-soil CO₂ and O₂ concentration and efflux values

The CO₂ concentrations in the treatment plots increased by about 35% for P. densiflora and about 50% for Q. variabilis seedlings immediately after the CO₂ was injected on 20 June and remained high during the CO₂ injection period from 20 June to 20 July (Figure 2). The CO₂ concentrations were reduced by 56.6% for P. densiflora and 26.1% for Q. variabilis on 18 July, and the difference in CO₂ concentrations between the control and treatment plots increased over time. On 5 and 19 July, there were significant differences between the CO₂ release period for the three species. The CO₂ concentrations in the control and treatment plots were reduced by 56.6% for P. densiflora and 26.1% for Q. variabilis on 18 July, and the difference in CO₂ concentrations between the control and treatment plots increased over time. On 5 and 19 July, there were significant differences between the CO₂ release period for the three species.

Variations in the Pn, Gs, and E of P. densiflora and Q. variabilis seedlings in both the control and treatment plots are shown in Figure 4. For both species, the Pn of the seedlings in the treatment plot was similar to that in the control plot before the CO₂ release (Figures 4a and 4b). The difference in Pn between the control and treatment plots began to increase after the initiation of CO₂ enrichment until the enrichment was stopped on 20 July. On 6 July, the Pn for the treatment plot (1.65 μmol m⁻² s⁻¹ for P. densiflora and 0.51 μmol m⁻² s⁻¹ for Q. variabilis) was significantly lower than that for the control plot (5.05 μmol m⁻² s⁻¹ for P. densiflora and 1.36 μmol m⁻² s⁻¹ for Q. variabilis) on 20 July. The biggest difference in Pn between the control and treatment plots was observed: lower by 50.8% for P. densiflora and 79.7% for Q. variabilis in the treatment plot than in the control plot. Gs and E for the treatment plots differed from those for the control for P. densiflora (Figures 4c and 4d). However, there was no significant difference in Gs and E between the control and treatment plots for Q. variabilis before, during, or after the injection of CO₂ gas (Figure 4e and 4f). For both the P. densiflora and Q. variabilis seedlings, the TDW values did not vary between the control and treatment plots (Table). The NL of the plants in the treatment plots (3121 ± 255 leaves for P. densiflora and 288 ± 52 leaves for Q. variabilis) was significantly higher than...
for the seedlings in the control plots (2697 ± 153 leaves for P. densiflora and 95 ± 4 leaves for Q. variabilis). In addition, MLA of P. densiflora and Q. variabilis seedlings was respectively 33% and 45% lower in the treatment plot than in the control plot due to the unusual leaf growth following the CO₂ release. The TLA of P. densiflora was much higher (0.24 ± 0.01 m²) in the control plot than it was in the treatment plot (0.19 ± 0.02 m²), whereas for Q. variabilis, the value was significantly higher in the treatment plot (0.35 ± 0.02 m²) than it was in the control plot (0.21 ± 0.02 m²).

3.3. Seedling growth
The total biomass of the P. densiflora seedlings differed between the control and treatment plots, with averages of 85.26 g tree⁻¹ and 66.15 g tree⁻¹, respectively (Figure 5). For Q. variabilis seedlings, the average biomass of seedlings in the control versus the treatment plots was approximately the same, with averages of 63.25 g tree⁻¹ and 69.10 g tree⁻¹, respectively. The biomass of the P. densiflora seedlings was primarily distributed in the leaf (46% and 43%) and stem (39% and 42%) in both the control and treatment plots, with relatively low biomass in the root area (15% for both the control and treatment plots). In contrast, in the control and treatment plots, biomass values for Q. variabilis seedlings of 63% and 58%, respectively, were concentrated in the root.

For the P. densiflora and Q. variabilis seedlings, the fine root (<2 mm) ratio in the treatment plots was higher than it was for the seedlings in the control plots (Figure 6a). The fine root percentage of the total root biomass of the plants in the control and treatment plots were 27.2% ± 1.2% and 39.8% ± 1.9%, respectively, for P. densiflora seedlings and 7.0% ± 0.9% and 11.0% ± 1.5%, respectively, for Q. variabilis seedlings. The in-soil O₂ concentration in the treatment plots decreased with rising in-soil CO₂ concentration and reached 0% when the CO₂ concentration reached 100% during the artificial CO₂ release experiment (Figure 6b).

3.4. Mortality
For P. densiflora seedlings, there was a 0% mortality rate for both control and treatment plots (Figure 7). For Q. variabilis seedlings, the mortality rate of the treatment plot was approximately 20% higher than that of the control plot (P < 0.01): 1.6% in the control plot and 20.8% in the treatment plot.

4. Discussion
4.1. Physiological and growth responses on elevated in-soil CO₂
A significant decline in Fv/Fm, which is correlated with photosynthesis efficiency, was observed when both P. densiflora and Q. variabilis seedlings were treated (Figures 3a and 3b). Environmental stress, including a high CO₂ concentration, can inhibit the ability of these species to metabolize normally, indicating an imbalance between the absorption of light energy in chlorophyll and the use of energy in photosynthesis (Chapman et al., 2015). This stress also reduced the total chlorophyll content in both species (Figures 3c and 3d). The decrease in the photosynthesis efficiency and the total chlorophyll content may be enhancing the problem with photosynthesis and be responsible for the inhibition of physiological function (Figures 4a and 4b) (Phan et al., 2007; He et al., 2016). The long-term accumulation of such damage will eventually affect the growth of both species (Austin et al., 1986).

3a and 3b). Environmental stress, including a high CO₂ concentration, can inhibit the ability of these species to metabolize normally, indicating an imbalance between the absorption of light energy in chlorophyll and the use of energy in photosynthesis (Chapman et al., 2015). This stress also reduced the total chlorophyll content in both species (Figures 3c and 3d). The decrease in the photosynthesis efficiency and the total chlorophyll content may be enhancing the problem with photosynthesis and be responsible for the inhibition of physiological function (Figures 4a and 4b) (Phan et al., 2007; He et al., 2016). The long-term accumulation of such damage will eventually affect the growth of both species (Austin et al., 1986).

The woody plant ecosystem can, either directly or indirectly, be affected by the leakage of geologically stored CO₂ and an increase in in-soil CO₂ (Kim et al., 2018a). In addition, despite relatively short periods of CO₂ release, the concentrations of in-soil O₂ dramatically decreased by up to 5% during the CO₂ release period (Figure 6b). Low levels of in-soil O₂ may result in asphyxia and mortality to woody plant seedlings, particularly the Q. variabilis seedlings (Figure 7) (Brooks et al., 2000). The short-term effects of elevated in-soil CO₂ significantly affected the physiological responses of P. densiflora and Q. variabilis seedlings. Thus, high levels of in-soil CO₂ can be stressful for P. densiflora and Q. variabilis seedlings by reducing the...
study, elevated in-soil CO₂ resulted in an increase in the heavy rain episode (Millard and Proe, 1992). In this have a second flush induced by a high-N condition or (Borchert, 1975). However, fixed-growth species can which all the components of the shoots in one year are of fixed-growth plants (Millard and Proe, 1992), for longevity, secondary metabolites, and high concentrations the plant to conserve resources for high leaf and root concentrations of in-soil O₂ (Vodnik et al., 2006; Bellante Q. variabilis P. densiflora species are representative of fixed-growth plants (Millard and Proe, 1992), for which all the components of the shoots in one year are commonly contained in the buds preformed the previous year, and the number of leaves and nodes is determined by the environmental conditions of the previous year (Borchert, 1975). However, fixed-growth species can have a second flush induced by a high-N condition or heavy rain episode (Millard and Proe, 1992). In this study, elevated in-soil CO₂ resulted in an increase in the number of leaves and the inhibition of leaf growth for both species (Table). Furthermore, leaves from the second flush appeared lighter green or even yellowish due to the low N compared to other normal leaves (data not shown). The relative decrease in the surface area of leaves allows the plant to conserve resources for high leaf and root longevity, secondary metabolites, and high concentrations of cell walls (Casper et al., 2001). However, this condition reduces the total carbon fixation from photosynthesis (Marron et al., 2003). In addition, the inhibition of leaf growth induces more leaves per tree to increase the total leaf area (Atta et al., 2015).

In agreement with this result, the functional imbalance resulting from a decrease in canopy photosynthesis is likely to increase the number of flushes and, eventually, the total number of leaves per tree (Table) (Borchert, 1975). The succession of flushes in a short time period has been observed in some fixed growth species with very high root/shoot ratios caused by increased water stress (Dostal, 1927). These observations imply that fixed growth species such as P. densiflora and Q. variabilis are capable of a second flush of growth in one season under conditions such as elevated in-soil CO₂, a long photoperiod, or sufficient moisture supply (Ronringer, 1963).

Furthermore, the percentage of fine root biomass increased when the total O₂ concentration decreased, resulting from the elevated in-soil CO₂ (Figures 5 and 6b). The carbon distribution between above and below the ground, which is determined by the amount of canopy photosynthesis, can be controlled by the type of species, environmental conditions, plant phenology, and external stress (Klepper, 1991). In particular, root growth has been known to be sensitive to an increase in in-soil CO₂ (Poozer, 1993). The relatively low in-soil O₂ obtained from the dilution effect of rising CO₂ concentration (Jones et al., 2014), may result in stress to the P. densiflora and Q. variabilis seedlings (Kim et al., 2018a). As a result, the seedlings of both species seem to distribute more carbon to the fine root compared to the coarse root to increase the availability of O₂ (Schulze, 1983; Ewett, 2004). Thus, forming fine roots is advantageous for accessing more in-soil CO₂ in the soil pores. Otherwise, some species such as Q. variabilis, which have a low percentage of fine roots, may the due to hypoxic stress (Figure 7) (Dale, 1985; Farrar and Gunn, 1996; Rogers et al., 1996).

Under ideal growing conditions, increasing carbon allocation to leaf tissue production leads to an increase in carbon uptake as well as carbon consumption due to leaf respiration (Dale, 1992; Davies, 1998). Carbon allocation towards root tissue production is essential for securing the essential resources needed for photosynthesis and growth, and lack of these critical resources increases carbon allocation to root tissue (Austin et al., 1986). The leaf growth and root function of P. densiflora and Q. variabilis seedlings was inhibited due to the increased CO₂ in the ground (Table). Therefore, the carbon allocation to leaves and fine roots increased, because it was difficult to acquire essential resources such as carbon, moisture, and oxygen (Schulze et al., 1987). These results indicate that the availability of essential resources can affect the carbon allocation needed to produce various tissues, and thus has a direct effect on plant survival, growth, and reproduction (Smith and Smith, 2015).

4.2. Species-specific responses to elevated in-soil CO₂ P. densiflora seedlings had significantly lower Gs and E in the treatment plot than they did in the control plot and may maintain a favorable water content and leaf water potential throughout the CO₂ release period (Figures 4c and 4e).

This relatively constant leaf water potential can be caused by decreased stomatal conductance as the stomata close (Jones and Tardieu, 1998). Consequently, water loss can be reduced for P. densiflora, but the risk of carbon starvation will increase (Figure 5) (Buckley, 2005). By contrast, Q. variabilis is a representative anisohydric species in which the leaf water potential decrease is correlated with the decrease in root water potential by maintaining stomatal conductance as the stomata are opened (Figures 4d and 4f) (Jones, 2007). As a result, Q. variabilis species will continue to transpire and assimilate carbon, even if severe water stress follows (Jones and Tardieu, 1998; Comstock, 2002). P. densiflora and Q. variabilis seedlings induce a second flush to increase the number of leaves and the total leaf area per tree, while elevated in-soil CO₂ may result in the inhibition of leaf growth (Table). On the other hand, P. densiflora seedlings have a low total leaf area and close stomata under a high concentration of in-soil CO₂ which reduces their biomass due to the lack of carbon assimilation (Figures 4 and 5). Q. variabilis seedlings, however, increased TLA and opened stomata under high in-soil CO₂ conditions to maintain their biomass despite the low net photosynthetic rate (Figures 4 and 5). These different stomatal behaviors may result from the different genotypes.

5. Conclusion
This study was conducted to investigate the effects of the leakage of underground CO₂ on surface woody plants by conducting a carbon capture and storage (CCS) project. The physiological and growth responses of P. densiflora and Q. variabilis seedlings were analyzed after an artificial CO₂ release at the EIT site. For both species, the photosynthesis efficiency and total content of chlorophyll were significantly reduced with the rise of the in-soil CO₂ which induced a decrease in the photosynthetic rate. On the other hand, this phenomenon effects on stomatal conductance and...
respiration varied according to species. The response of *P. densiflora* was to close its stomata. This reduces water stress but may result in carbon starvation. The stomata of *Q. variabilis* opened, which allowed an increase in carbon fixation but also induced water loss. These results indicate that the physiological and growth responses of plants to the elevated and in-soil CO₂ is species-specific. Overall, it appears that reduced physiological functions might be related to the O₂ depletion in the soil pores which might have been driven away by enriched CO₂.

Relatively short-term CO₂ release affected carbon allocation between coarse and fine roots. The short-term CO₂ release affected carbon allocation between coarse and fine roots and possibly effects of hypoxia must be analyzed more thoroughly by species in further research.

Acknowledgments

This study was supported by the Environmental Management of Geologic CO₂ Storage Project (2014001810002) of the KO-SEOM Research Center, which was funded by the Korea Ministry of Environment. It also received help from the Thinning Demonstration Research (NRF-2018R1D1A1B07042483) funded by National Research Foundation of Korea, and from a Korea University Grant.

References

Attila Z, Domec J-C, Oren R, Way DA, Moshelion M (2015). Growth stress but may result in carbon starvation. The stomata of *Q. variabilis* opened, which allowed an increase in carbon fixation but also induced water loss. These results indicate that the physiological and growth responses of plants to the elevated and in-soil CO₂ is species-specific. Overall, it appears that reduced physiological functions might be related to the O₂ depletion in the soil pores which might have been driven away by enriched CO₂.

Relatively short-term CO₂ release affected carbon allocation between coarse and fine roots. The short-term CO₂ release affected carbon allocation between coarse and fine roots and possibly effects of hypoxia must be analyzed more thoroughly by species in further research.


