

Physiological responses of dandelion and orchard grass leaves to experimentally released upwelling soil CO₂



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ABSTRACT

Carbon sequestration is an important means of reducing the concentration of atmospheric CO₂ by injecting CO₂ into subterranean geological reservoirs known as sequestration fields. These deep sequestration fields must be monitored for structural integrity to ensure their long-term efficacy. Stress responses of plants to super-elevated soil CO₂ are signatures that we evaluated as potential tools for surface leak detection of CO₂ at the Zero Emission Research and Technology (ZERT) site in Bozeman, MT. To mimic a compromised field, CO₂ was deliberately released at a rate of 0.15 ton/day through a 100 m long and 2.0–2.3 m deep Horizontal Injection Well (HIW) with intentional leaks, with injections of CO₂ taking place on these dates: 7/19–8/15/2010 and 7/18–8/15/2011. Periodically over two years, we measured stomatal conductance, chlorophyll content, and specific leaf area in *Taraxacum officinale* Wigg. (dandelion) and *Dactylis glomerata* L. (orchard grass) along a 20 m transect perpendicular to a hot spot. Hot spots were visible as circular zones of leaf dieback where *T. officinale* leaves turned from green to red and became brown and desiccated. CO₂ concentrations reached 29% by volume at the edges of hot spots. During CO₂ injection, chlorophyll content (as measured with a Hansatech CL-O1 chlorophyll content meter to obtain a unitless measure) decreased significantly ($p < 0.001$) in *T. officinale* from 9.486 to 1.912 (80%) in 2010 and from 8.288 to 0.000 (99%) in 2011. The less pronounced decreases in chlorophyll content with CO₂ injection in *D. glomerata* were 34% in 2010 and 37% in 2011. Average stomatal conductance (g_s) rates in *T. officinale* were highest at the hot spot (22.41 mmol m⁻² s⁻¹ in 2010, 46.10 mmol m⁻² s⁻¹ in 2011) and lowest 20 m distally (11.76 mmol m⁻² s⁻¹ in 2010 and 3.69 mmol m⁻² s⁻¹ in 2011). Average g_s rates in *D. glomerata* were highest at the hot spot (53.91 mmol m⁻² s⁻¹ in 2010 and 65.96 mmol m⁻² s⁻¹ in 2011), and lowest 20 m distally (38.52 mmol m⁻² s⁻¹ in 2010 and 24.83 mmol m⁻² s⁻¹ in 2011). These results suggest *T. officinale* leaves are more sensitive to super-elevated soil CO₂ concentration than *D. glomerata* leaves and generally, that species-specific physiological responses of these leaves to varying concentrations of soil CO₂ may be useful in monitoring CO₂ leakage in vegetated areas above CO₂ storage sites.

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1. Introduction

One important measure toward reducing the concentration of CO₂ in the earth's atmosphere is the storage of CO₂ in deep geological formations such as depleted oil or gas fields and deep saline formations (Metz et al., 2005). Carbon sequestration sites must be stringently monitored in order to detect possible leakage of

CO₂ and to ensure successful sequestration. Plants exhibit stress symptoms when exposed to super-elevated soil CO₂ concentrations such as those which might be encountered during acute leaks from sequestration fields, and therefore plants are valuable in the surface detection of CO₂ (Male et al., 2010; Lakkaraju et al., 2010). The Zero Emission Research and Technology (ZERT) site in Bozeman, MT is an experimental carbon release field facility designed to study the effects of leaking and accumulating CO₂ on soil and vegetation and to develop methods for the surface detection of CO₂ (Spangler et al., 2009). At the ZERT site, controlled shallow releases of CO₂ reached concentrations of greater than 20% (the maximum

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calibrated value we can measure) while upwelling through the soil and rhizosphere to the aboveground parts of herbaceous plants, including *Taraxacum officinale* (dandelion) and *Dactylis glomerata* (orchard grass). The CO₂ release experiments at the ZERT site are characterized by the formation of highly visible circular zones of pronounced leaf dieback, known as hot spots, which form above the zones of upwelling CO₂ (Male et al., 2010; Lakkaraju et al., 2010).

The ZERT site is a valuable facility for monitoring responses of plants to the super-elevated soil CO₂ associated with leaking CO₂. High concentrations of leaking and accumulated soil CO₂ (Hepple and Benson, 2005; Patil et al., 2010) rapidly result in visible stress symptoms in plants at the ZERT site (Male et al., 2010; Lakkaraju et al., 2010). High soil CO₂ can cause stress in plants by inhibiting root respiration and the uptake of nutrients and water (Macek et al., 2005), and aerenchyma became more extensive with elevated CO₂ in root of *Zea mays* L. (Videmsek et al., 2006). High soil CO₂ resulted in decreased height, chlorophyll content and dry weight in *Z. mays* (Vodnik et al., 2005; Noomen and Skidmore, 2009) and it hindered germination and halved the standing biomass of winter beans (Patil et al., 2010). In a natural system, trees died with exposure to CO₂ gassing from volcanic vents at Mammoth Mt., California (Rogie et al., 2001). While investigations of leaf-level responses to elevated atmospheric CO₂ are abundant (Kelly et al., 1991; Wheeler et al., 1999; Apple et al., 2000; Woodward et al., 2002; Levine et al., 2008), plants at the ZERT experiment were exposed to super-elevated soil CO₂ in the context of surface detection of leaking CO₂ from sequestration fields. Changes in spectral signatures and leaf reflectance have been found in response to super-elevated soil CO₂ at mofettes and at experimental sequestration fields (Vodnik et al., 2005; Noomen and Skidmore, 2009; Lakkaraju et al., 2010; Male et al., 2010; Bellante et al., 2013), as well as elevated atmospheric CO₂ (Galvencio et al., 2011), and here we investigate the response of physiological parameters of individual leaves to super-elevated soil CO₂ concentrations at an experimental carbon sequestration field.

Physiological stress responses of plants to elevated atmospheric CO₂ include changes in stomatal conductance, pigmentation, and fluorescence (Field et al., 1995; Ge et al., 2011; Nability et al., 2012). Stomata adjust between opening to allow uptake of atmospheric CO₂ for carbon fixation and closing to limit water loss during transpiration. Although plants generally respond to elevated atmospheric CO₂ by decreasing stomatal conductance (Wand et al., 1999; Medlyn et al., 2001; Wullschlegel et al., 2002; Long et al., 2004; Ainsworth and Long, 2004), stomatal conductance increased in potato, soybean and sweet potato with very high or super-elevated atmospheric CO₂ (5000–10,000 $\mu\text{mol mol}^{-1}$) (Wheeler et al., 1999). Stomatal conductance decreased in soybeans with exposure to 1200 $\mu\text{mol mol}^{-1}$ CO₂ but increased with 10,000 $\mu\text{mol mol}^{-1}$ CO₂ (Levine et al., 2008). Chlorophyll content decreases with plant stress and senescence (Gitelson and Merzlyak, 1994) and has been associated with increased carotenoid concentration (Lakkaraju et al., 2010). Changes in chlorophyll content are useful indicators of stress, photosynthetic capacity, primary productivity, and leaf nitrogen content (Curran et al., 1990; Filella et al., 1995; Moran et al., 2000). Measurements of chlorophyll fluorescence are useful in evaluations of plant stress since proportionately more of the energy absorbed by chlorophyll dissipates as fluorescence than is normally channeled into photochemistry when plants experience abiotic stress (Kalaji and Guo, 2008).

The ZERT site is located in a former agricultural field where the vegetation reflects this previous use, as it consists of a mixture of grasses, legumes, and other herbaceous plant species, of which, *T. officinale* (dandelion) and *D. glomerata* (orchard grass) are codominant. At the ZERT site, we established a transect along a gradient of decreasing soil CO₂ concentration that ranged from a hot spot to a control site. Before and during CO₂ injections, we measured

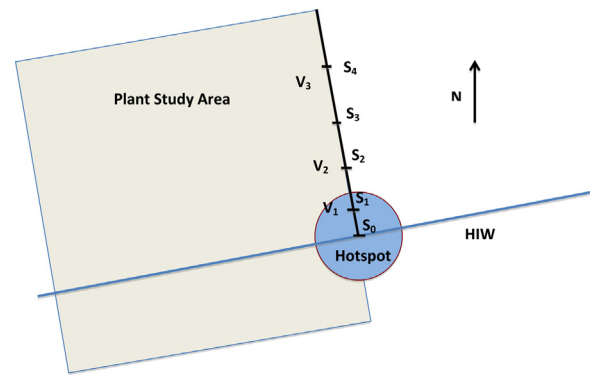


Fig. 1. The ZERT plant study area. In 2010, the transect was divided into 4 positions, or stations, (S₁–S₄), with S₁ approximately 1 m away from the site of CO₂ injection at the Horizontal Injection Well (HIW). S₄, the control, was approximately 7 m away from the pipe. S₀ was added in 2011. Vaisala CO₂ probes were deployed at V₁–V₃ and the approximate location of the hotspot of CO₂ leakage is indicated by the red circle.

soil CO₂; the leaf area index of the vegetation; and stomatal conductance, chlorophyll content, fluorescence, and specific leaf area in individually tagged *T. officinale* and *D. glomerata* leaves. This was done in order to determine whether changes in these particular plant stress responses may prove to be important tools in surface detection of leaking and accumulating soil CO₂ at the experimental ZERT site; at actual carbon sequestration fields; and as a means of augmenting existing information on plant stress responses at naturally occurring CO₂ vents.

2. Materials and methods

2.1. Field design and plants

The ZERT site is situated in a former agricultural field at Montana State University in Bozeman, MT, where a 100 m Horizontal Injection Well (HIW) for the controlled release of CO₂ runs SW to NE at a depth of approximately 2.0–2.3 m, roughly parallel to and beneath the ground surface (Spangler et al., 2009). The HIW is divided into six independent zones with packers. The flow rate was controlled with a mass flow controller in each zone. Leaking CO₂ upwells from each zone during the experimental injections of CO₂ into the HIW. From 7/19–8/15/2010 and from 7/18–8/15/2011, CO₂ was released through the HIW at 0.15 ton/day.

An approximately 10 m × 10 m plant research area within the ZERT site extended northwest of the HIW. Within the plant research area, we established a 10 m long transect perpendicular to the HIW and parallel to one edge of the plant research area. In 2010, the positions along the transect at which measurements were carried out and their distances northwest of the HIW were as follows: Station 1 (S₁) one meter, Station 2 (S₂) three meters, Station 3 (S₃) five meters, and Station 4 (S₄) seven meters. In 2011, we added Station 0 (S₀) at the intercept of the transect and the HIW (Fig. 1). Plants were not fertilized, irrigated, trampled or mowed during the experiments. The ZERT site vegetation consists of pre-existing plants which were not specifically planted for the experiments. Naturally occurring grasses and other herbaceous plants at the ZERT site include dandelion, *Taraxacum officinale* Wigg., and orchard grass, *Dactylis glomerata* L., as codominant species, along with Canada thistle, *Cirsium arvense* (L.) Scop.; alfalfa, *Medicago sativa* L.; birds-foot trefoil, *Lotus corniculatus* L.; yellow blossom sweet clover, *Melilotus officinalis* (L.) Pall.; red clover, *Trifolium pratense* L.; lupine, *Lupinus argenteus* Pursh; quackgrass, *Elymus repens* (L.) Gould; timothy, *Phleum pratense* L.; Kentucky bluegrass, *Poa pratensis* L.; field brome, *Bromus arvensis* L.; and smooth brome, *Bromus inermis* L.

2.2. Soil CO₂ concentration

Soil CO₂ concentration at a depth of 15 cm in 2010 and 20 cm in 2011 was measured with Vaisala 221 GMT probes (with a calibrated range of 0–20% volume) with adaptors for vertical positioning under the soil at each station along the transect in 2010 and 2011. The probes ran in all weather. Data was averaged and stored every 5 min.

2.3. Leaf physiology

We measured leaf area index (LAI), (the sum of the projected leaf surface area per soil area, or, m² foliage area/m² ground area), in 2009 with a LAI-2200 (Li-Cor, Inc.), along the plant study transect.

The timing of visible leaf color change and leaf dieback during the CO₂ injections and subsequent hot spot formation was noted and photographed. Chlorophyll content, stomatal conductance, and fluorescence were measured *in-situ* at the mid-point, or center, of randomly selected, fully expanded and labeled dandelion and orchard grass leaves ($n = 12$ leaves per station in 2010 and $n = 10$ leaves per station in 2011, and $n = 5$ for new dandelion leaves emerging after injection per station in 2011). Forty-eight leaves per species were measured repeatedly in 2010, and 50 leaves per species were measured in 2011. Relative chlorophyll content (0–2000 units) determined via dual wavelength optical absorbance at 620 and 940 nm was measured with a chlorophyll content meter CL-O1 (Hansatech, Inc.), approximately every other day on 16 different days in 2010, with 1 measurement day before, 14 measurement days during, and 1 measurement day after the CO₂ injection. Chlorophyll content was measured on 7/16 (pre-injection), and on 7/20, 7/25, 8/1, 8/4, 8/6 and 8/10 in 2011.

Stomatal conductance (g_s) of the abaxial surfaces of dandelion and orchard grass leaves was measured at mid-leaf under mid-day ambient conditions with a Decagon SC-1 steady state porometer that simultaneously recorded temperature (5–40 °C) and stomatal conductance with an operating range of 0–90% RH on 20 days in 2010: 2 before, 17 during, and 1 after the CO₂ injection, and at approximately two day intervals from 7/15–8/10/2011 while leaves were available for measurement. Atmospheric CO₂ values immediately surrounding the leaves were not available, but soil CO₂ at the hot spot (S_1) reached 29%, 5% at S_2 , and 0.90% at S_4 (control). Relative humidity in midday sunlight averaged 50% in 2010 and 37.8% in 2011. By 8/4/2010, most of the initial cohort of dandelion leaves at S_1 had died and we then measured g_s in small green dandelion leaves that emerged at S_1 after CO₂ injection. Although the 2011 injection lasted until 8/15/2011, we discontinued stomatal measurements due to leaf dieback at S_0 and S_1 by 8/5/2011. The ratio (in percentage) of stomatal conductance (g_s) at station 1 (S_1) to that at station 4 (S_4) for any given date was calculated as follows:

$$\frac{g_s(S_1)}{g_s(S_4)} \times 100\%.$$

Since S_4 was the control site, the ratio was used to compare the relative change of stomatal conductance at S_1 with that of S_4 to emphasize the effect due to soil CO₂ than due to natural senescence.

Chlorophyll fluorescence, as F_v/F_m (which is the exciton transfer efficiency in dark adapted leaves and is derived from $(F_m - F_o)/F_m$, where F_m = maximum fluorescence in dark-adapted tissue; F_o = minimal fluorescence in dark-adapted tissue), was measured with an FMS-2 modulated fluorometer (Hansatech, Inc.) from 7/19–8/15/2010 and from 7/18–8/10/2011 at mid-leaf in ten-dark adapted dandelion leaves per station. Dandelion leaves measured were sufficiently wide enough to cover the sensor.

We collected dandelion leaves ($n = 10$ per station) before and after CO₂ injection in 2011, measured them with a leaf area meter

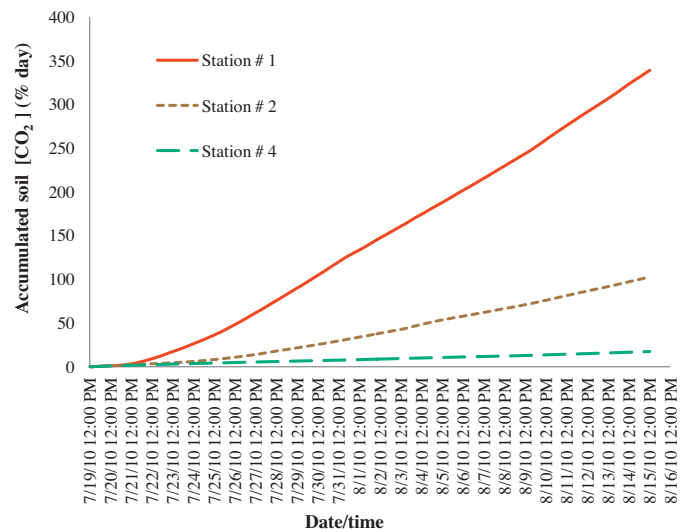


Fig. 2. Accumulated soil concentration from measured % volume CO₂ concentrations during the 2010 experimental injection at S1, S2, and S4. Accumulated soil CO₂ concentrations was calculated from the beginning of the CO₂ release as % day (equal to the total exposure to 1% soil CO₂ concentration for 24 h).

(CID-Inc.), determined their dry weights, and calculated specific leaf area (cm²/g dry weight) for each individual leaf.

The percentage of N, P, and K in two samples ($N = 10$ leaves/sample) of dandelion and one sample ($N = 10$ leaves/sample) of dry ashed orchard grass leaves was determined via CHN analysis and ICP-OES (Inductively Coupled Plasma/Optical Emission Spectrometry).

2.4. Statistical analyses

Measurements of physiological parameters in dandelion and orchard grass leaves were tested for significant differences with a paired *t*-test to calculate *p* values, with significance set at $p \leq 0.05$ (Minitab, SAS, and Prism), between stations along the transect and before and after exposure to super-elevated soil CO₂. Linear regression was used to yield *r* values (*r*) with distance from the Horizontal Injection Well, the source of super-elevated soil CO₂.

3. Results

3.1. Soil CO₂

In 2010, the overall concentrations of soil CO₂ ranged from 1% to 16% by volume; with 1–16% at S_1 , (1 m from the HIW), 1–5% at S_2 (3 m from the HIW), we did not deploy a sensor at S_3 , and 0.5–0.9% at S_4 , the designated control with a normal (0.5–2%) range of CO₂ (Russel, 1973). In 2011, soil CO₂ was 0.841% by volume prior to injection and increased to 29% by volume at S_1 during the injection. At S_4 , initial soil CO₂ was 1.02% per volume with 0.10–0.90% per volume during the injection. Data collection at S_2 and S_3 was precluded by datalogger failure in 2011. Accumulated soil CO₂ was calculated from the beginning of the CO₂ release as % day (1% day is equal to the total exposure to 1% soil CO₂ concentration for 24 h) from measured % volume CO₂ concentrations (Fig. 2). The accumulated CO₂ exposure from the start of CO₂ release until a specific day is calculated from the sum of the products of soil CO₂ concentration and the total elapsed time in days (Lakkaraju et al., 2010).



Fig. 3. Hotspot of CO₂ leakage with leaf dieback during the 7/19–8/15/2010 injection, photographed on 8/5/2010 following 18 days of CO₂ leakage.

3.2. Hot spots

Circular hot spots of leaf senescence and dieback with an approximate 3 m diameter formed within one to two weeks of CO₂ injection. The hot spots had dead, often brown leaves and a thinner canopy cover than at the control station (S₄). Hot spot locations corresponded with zones of maximum soil CO₂ concentrations above the HIW (Fig. 3). At the hot spots, all dandelion leaves present before CO₂ injection changed from green to reddish purple to a desiccated brown, signifying their dieback (Fig. 4). Reddish purple leaves indicated increased anthocyanin content. Color change and leaf dieback followed the CO₂ concentration gradient and were most rapid and pronounced closest to the HIW. Colors at S₁ changed within one week of CO₂ release in 2010, and within 3–4 days in 2011. Leaves died within two weeks of CO₂ release (by 8/2/2010 and by 7/31/2011). Color change was minimal at S₄. After the initial cohort of dandelion leaves senesced, very small (3–4 cm long) green dandelion leaves emerged at S₁ by 8/4/2010, but their chlorophyll content decreased significantly thereafter. A similar cohort of new green dandelion leaves lost chlorophyll after emerging during the CO₂ injection in 2011.

Orchard grass leaves did not change color rapidly or senesce, but gradually changed from early-season green to straw-yellow. At S₁, this occurred within three weeks of CO₂ injection in 2010 and within two weeks in 2011. Orchard grass at S₂ did not turn yellow until the injection ended in 2010, and not until the third week in



Fig. 4. Dandelion leaves on 8-3-10 at: a. (S₁), hot spot, and b. (S₄), control. Leaves at S₁ typically had this appearance following exposure to CO₂, while the leaves at S₄ remained green throughout the experiment.

2011. Orchard grass did not have a pronounced color change at S₃ or S₄ other than those that are subtle preludes to their normal, late season senescence.

3.3. Leaf area index (LAI)

At S₁, LAI decreased from 2.67 on 7/14/09 to 1.16 on 7/28/09, when leaf dieback had occurred at S₁ but not at S₄, where LAI decreased from 4.22 on 7/14/09 to 3.43 by 7/28/09.

3.4. Specific leaf area (SLA)

Post-injection SLA values (cm²/g dry weight) were significantly higher than pre-injection values ($p=0.0011$), but they did not increase significantly with increased soil CO₂ concentrations (Table 1). Post-injection dandelion leaves were significantly ($p=0.0035$) smaller than pre-injection leaves at all stations, but did not differ significantly in area with CO₂ concentrations. One exceptionally large post-injection leaf at S₄ had an area of 67 cm², which by far exceeded the 9.66 cm² S₄ average. The initial cohort of leaves was approximately 75 days old at harvest on 7/16/11 and emerged in spring, 2011, approximately two months prior to the CO₂ injection. The smaller, post-injection leaves emerged in late summer and early fall; after the CO₂ injection.

3.5. Nutrient status of leaves

The percentages of N, P, and K in dandelion and orchard grass leaves indicated sufficient nutrients. Dandelion averages were 1.93% N; 0.50% P; and 5.63% K. Orchard grass averages were 0.95% N; 0.31% P; and 2.43% K.

3.6. Chlorophyll content of leaves

Chlorophyll content decreased significantly ($p \leq 0.05$) in dandelion and orchard grass leaves with exposure to accumulated soil CO₂ (percent day) in 2010–2011. Chlorophyll values for S₃ were not included here because there was no CO₂ sensor at S₃. In 2010, dandelion chlorophyll content decreased significantly with date and CO₂ accumulation close to the HIW at S₁ ($r=0.887$, $p=0.005$) and S₂ ($r=0.915$, $p=0.000$), but not distally at S₄ ($r=0.629$, $p=0.09$), and in orchard grass, chlorophyll content decreased significantly at S₁ ($r=0.916$, $p=0.00$), S₂ ($r=0.581$, $p=0.018$), and S₄ ($r=0.689$, $p=0.03$). The decrease at S₁ and S₂ may reflect the impact of CO₂ and seasonal senescence, while the decrease at S₄ may reflect seasonal senescence alone. In 2011, dandelion chlorophyll content decreased significantly with date and accumulated CO₂ at S₀ ($r=0.88$, $p=0.00$), at S₁ ($r=0.87$, $p=0.00$) and at S₂ ($r=0.59$, $p=0.00$). Orchard grass had significantly less chlorophyll at positions S₀ ($r=0.57$, $p=0.00$), S₁ ($r=0.52$, $p=0.00$), and S₂ ($r=0.44$, $p=0.00$) but not at S₄ ($r=0.02$, $p=0.78$), (Fig. 5a and b). Chlorophyll content decreased significantly in new dandelion leaves emerging at S₀ after the injection was initiated but before its completion in 2011 (Fig. 6).

3.7. Stomatal conductance

The rate of stomatal conductance (g_s), (mmol m⁻² s⁻¹) was significantly higher ($p \leq 0.05$) with the highest CO₂ concentrations close to the HIW (Fig. 7a and b). While atmospheric CO₂ concentrations immediately surrounding the leaves were unavailable, the super-elevation of soil CO₂ near the HIW suggests that atmospheric CO₂ was proportionately high, and leaf responses to the atmospheric CO₂ were likely in the absence of wind. Stomatal conductance was measured on the abaxial surface in the midday sun

Table 1
Specific leaf area (cm²/g dry weight) and leaf area (cm²) in dandelion leaves at ZERT in 2011.

Dandelion leaves				
Station	SLA (cm ² /g dry weight)		Leaf area (cm ²)	
	Pre-injection	Post-injection	Pre-injection	Post-injection
S ₀	185.99 ± 40.18	245.28 ± 32.42	41.27 ± 15.48	9.60 ± 4.77
S ₁	171.77 ± 39.75	251.33 ± 27.10	58.91 ± 13.92	11.38 ± 4.64
S ₂	185.66 ± 57.56	297.60 ± 68.78	91.79 ± 43.48	5.26 ± 1.84
S ₃	184.36 ± 62.34	246.82 ± 26.23	83.03 ± 22.85	10.10 ± 5.44
S ₄	225.77 ± 126.48	312.65 ± 109.29	71.83 ± 38.45	15.68 ± 17.17

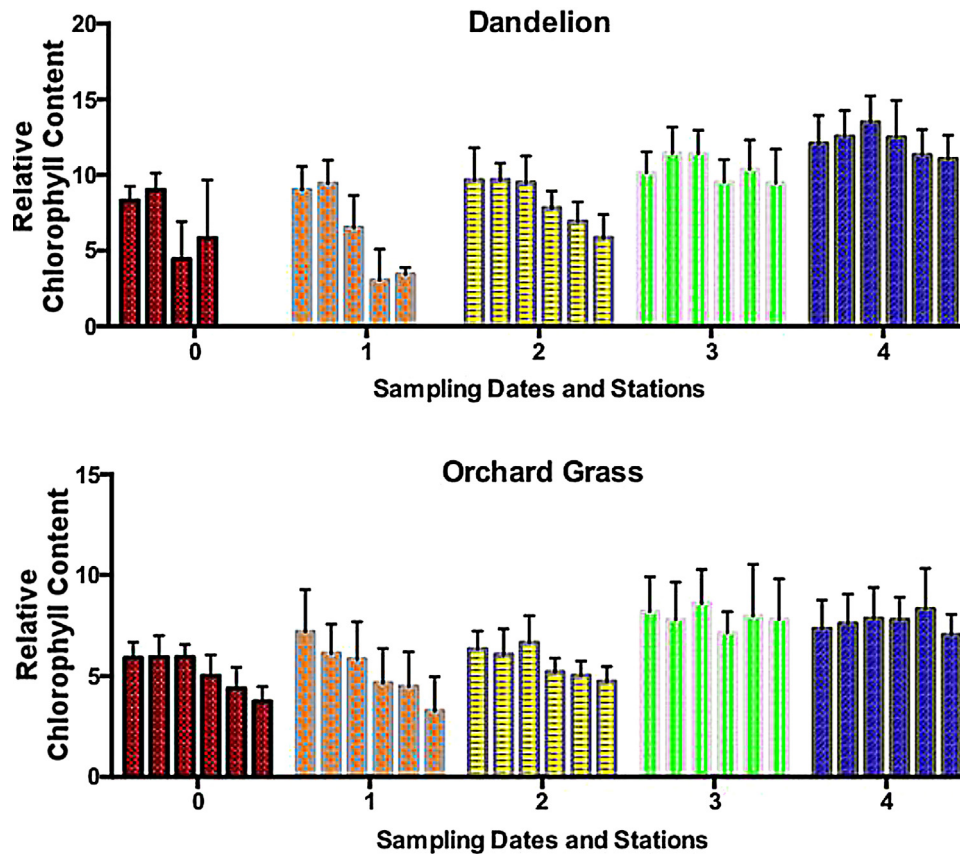


Fig. 5. (a and b) Decreases in chlorophyll content of dandelion and orchard grass leaves were most pronounced close to the hotspot of upwelling CO₂, with a greater decrease in dandelion leaves. The columns correspond to the following dates in 2011: 7/16 (pre-injection), 7/20, 7/25, 8/1, 8/4, and 8/10. Chlorophyll content in dandelion leaves was curtailed at S₀ after 8/1/11, due to the dieback of the initial cohort of dandelion leaves.

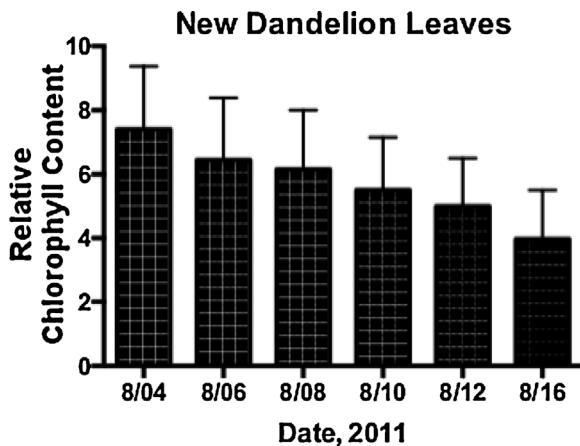


Fig. 6. Chlorophyll content decreased in dandelion leaves emerging at S₀ after CO₂ injection (n = 5).

for both amphistomatous species. Stomatal distribution was determined via observation of transverse leaf sections with a Nikon Labophot at 100×.

In 2010, stomatal conductance was significantly higher in dandelion leaves at S₁, (CO₂ from 1 to 18%), than at S₄, (CO₂ from 0.5 to 0.9%) for all dates. The average g_s rates over all experimental dates were: 22.41 ± 7.43 at S₁, 17.17 ± 6.13 at S₂, 11.71 ± 3.07 at S₃, and 11.76 ± 3.42 at S₄. The average leaf temperature was 33.43 °C, with an average of 33.59 ± 2.67 °C at S₁, 33.58 ± 2.59 °C at S₂, 33.70 ± 2.34 °C at S₃, and 32.89 ± 2.3 °C at S₄. In 2011, dandelion stomatal conductance was significantly higher at S₀ and S₁ than at S₂, S₃, and S₄. In 2011, the average g_s rates (mmol m⁻² s⁻¹) over all experimental dates were: 46.106 ± 22.35 at S₀, 30.689 ± 19.99 at S₁, 4.12 ± 2.22 at S₂, 3.79 ± 2.08 at S₃, and 3.69 ± 1.80 at S₄, with an overall average temperature of 32.41 °C, and an average of 33.13 ± 2.62 °C at S₀; 32.12 ± 2.68 °C at S₁; 32.49 ± 3.50 °C at S₂; 33.04 ± 2.87 °C at S₃, and 31.28 ± 2.70 °C at S₄. All initial dandelion leaves at S₀ died by 8/2/2011, and by 8/5/2011 at S₁, but those at S₂, S₃, and S₄ lived throughout the experiment.

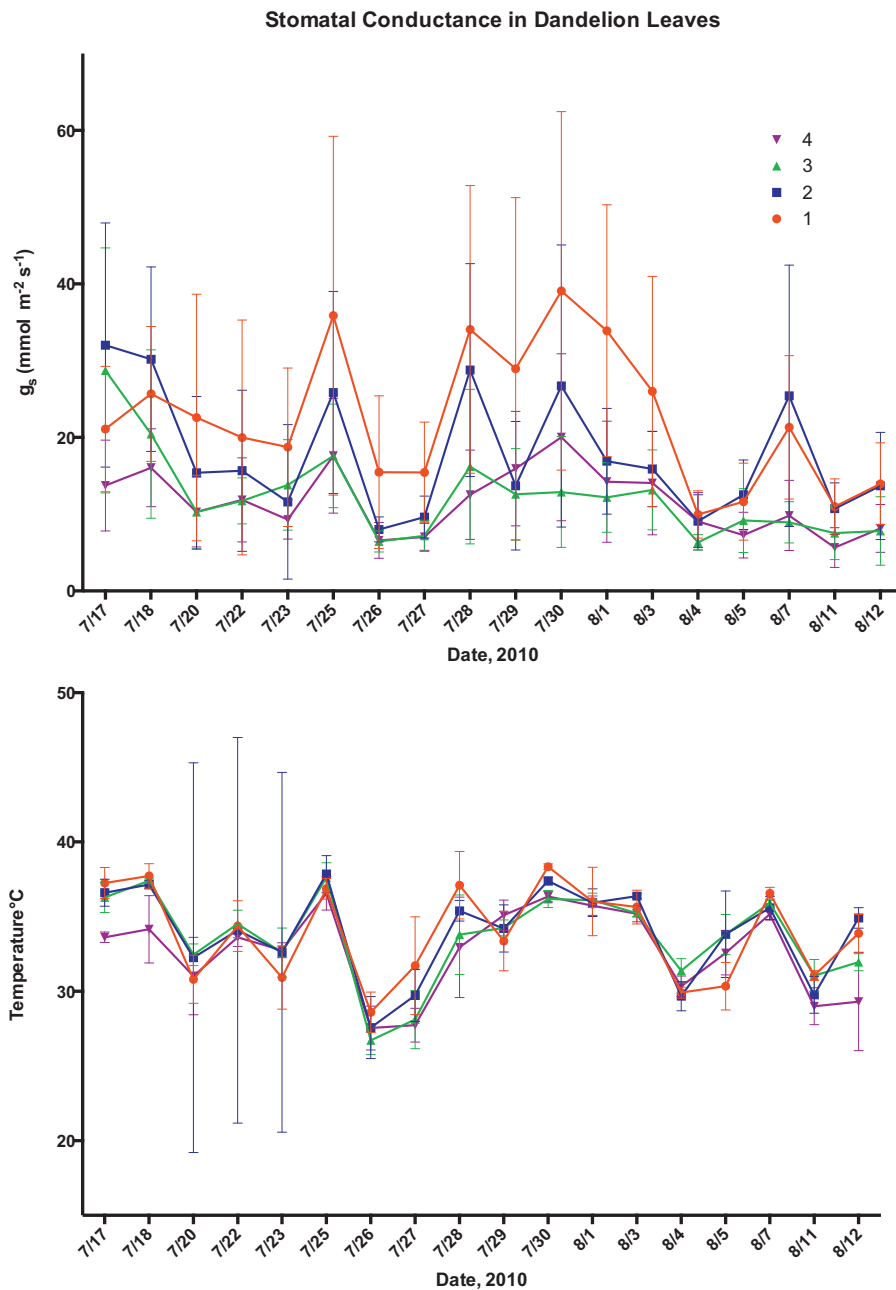


Fig. 7. (a and b) Stomatal conductance (g_s), in $\text{mmol m}^{-2} \text{s}^{-1}$, of dandelion leaves (a), was significantly higher at S_1 with elevated CO_2 ; (b) temperatures during g_s measurements. Station 1 (S_1) was closest to the Horizontal Injection Well (HIW), and Station 4 (S_4) was close to nominal ambient CO_2 concentrations.

In 2010, stomatal conductance of orchard grass leaves was significantly higher at S_1 than at S_4 except for 7/23 and 7/26 (Fig. 8a and b). Averaged over all 2010 experimental dates, g_s rates ($\text{mmol m}^{-2} \text{s}^{-1}$) were: 53.91 ± 22.08 at S_1 , 42.98 ± 15.42 at S_2 , 40.03 ± 17.19 at S_3 , and 38.52 ± 15.58 at S_4 ; with an overall average temperature of 32.41°C , and an average of $34.01 \pm 2.93^{\circ}\text{C}$ at S_1 , $33.35 \pm 2.76^{\circ}\text{C}$ at S_2 , $33.34 \pm 2.86^{\circ}\text{C}$ at S_3 , and $31.87 \pm 2.49^{\circ}\text{C}$ at S_4 . In 2011, stomatal conductance of orchard grass leaves was highest at S_0 and S_1 when compared to S_4 over all dates. Orchard grass g_s rates ($\text{mmol m}^{-2} \text{s}^{-1}$) for 2011 were: 65.96 ± 18.93 at S_0 , 51.97 ± 20.70 at S_1 , 28.89 ± 11.30 at S_2 , 35.28 ± 14.24 at S_3 , and 24.83 ± 9.5 at S_4 , with an overall average temperature of 32.79°C ; $34.56 \pm 2.85^{\circ}\text{C}$ at S_0 , $33.22 \pm 2.94^{\circ}\text{C}$ at S_1 , $32.43 \pm 3.04^{\circ}\text{C}$ at S_2 , $32.52 \pm 2.74^{\circ}\text{C}$ at S_3 , and $31.24 \pm 2.83^{\circ}\text{C}$ at S_4 .

Stomatal conductance was consistently and significantly higher in orchard grass leaves than in dandelion leaves over all dates at all stations (Fig. 9) and stomatal conductance increased significantly with increased CO_2 and temperature for both species.

3.8. Fluorescence

F_v/F_m was not significantly different with position in 2010, with average values at S_1 of 0.44 ± 0.06 ; at S_2 of 0.43 ± 0.09 ; at S_3 of 0.43 ± 0.07 ; and at S_4 of 0.456 ± 0.07 . These values indicate some photochemical stress at all positions, as F_v/F_m values of 0.79–0.84 are optimal (Maxwell and Johnson, 2000). In 2011, F_v/F_m was not significantly different with position early in the injection event, but later F_v/F_m values could not be determined because the initial cohort of dandelion leaves had died at S_0 and S_1 by 8/5/11.

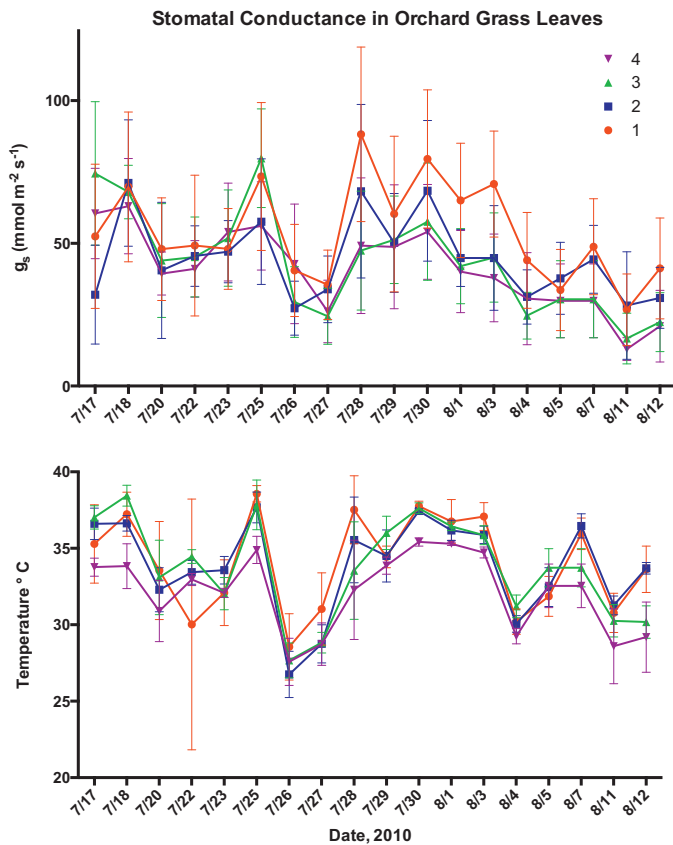


Fig. 8. (a and b) Stomatal conductance (g_s), in $\text{mmol m}^{-2} \text{s}^{-1}$, of orchard grass leaves (a), was significantly higher at S_1 with elevated CO_2 ; (b) temperatures during g_s measurements. Station 1 was closest to the Horizontal Injection Well (HIW), and Station 4 was close to nominal ambient CO_2 concentrations.

4. Discussion

Plants at the ZERT site responded visibly to upwelling CO_2 . The appearance of the hot spots within one to two weeks of CO_2 exposure along with the associated and likely accelerated leaf senescence within the hot spots are important, visually apparent signatures that can be readily used as tools in the surface detection of leaking CO_2 . The rapid leaf senescence within the hot spots is indicative of profound physiological responses of plants to the

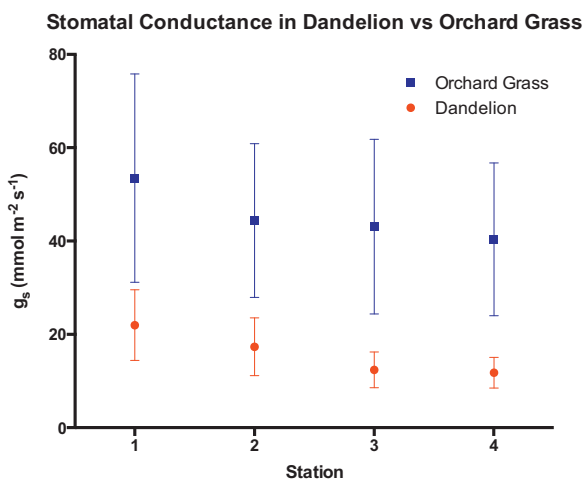


Fig. 9. Stomatal conductance was higher in orchard grass than in dandelion leaves with combined dates by station in 2010. Station 1 was closest to the Horizontal Injection Well (HIW), and Station 4 was close to nominal ambient CO_2 concentrations.

super-elevated soil CO_2 that occurs at the sites of experimentally leaking CO_2 at the ZERT site.

Dandelion leaves exhibited visible stress symptoms consisting of pronounced color changes from green to purple-red before senescing to a desiccated brown. Color changes were visible with super-elevated soil CO_2 within a week of the experimental injections in both 2010 and 2011. In addition to being measured as decreases in chlorophyll content, leaf stress was also detected by hyperspectral changes in leaf pigments (Lakkaraju et al., 2010); detectable at exposure levels as low as 4–8% CO_2 by volume (Male et al., 2010). The CO_2 concentrations where plant stress becomes apparent are well below the very high percentages of soil CO_2 found with the experimentally induced leak in CO_2 at the ZERT site. Visible stress responses in plants can also be valuable in the detection of natural gas leaks, where the reflectance spectra of stress-induced color changes from green to purple in oilseed rape leaves varied with exposure to different stresses (Smith et al., 2005). The reflectance spectra of oilseed rape leaves following exposure to extreme shade differed from those obtained following exposure to herbicides or natural gas, where the spectra were similar but appeared more quickly in response to natural gas (Smith et al., 2005). Thus, responses of plants can serve as valuable indicators of leaking CO_2 from experimental, industrial, and natural sites but timing, spectral signatures, and contexts are important in determining the cause of stress responses with similar appearances.

Oxygen is displaced from the soil by CO_2 , which then inhibits mitochondrial (Gonzalez-Meler et al., 1996) and thus root respiration (Palta and Nobel, 2006; Qi et al., 1994). CO_2 levels in the gaseous phase of the soil at the ZERT site exceeded five percent, and while O_2 concentrations at the ZERT site in 2010 did not decrease to the critical levels of 12–14% suggested by Adamse et al. (1972), as necessary for a healthy root system, they still decreased from an average of 19.060–21.180% at the control site to 18.220–20.690% at the hot spot (Zhou et al., 2013). However, elevated CO_2 not only displaces O_2 , but it can also enhance evaporation and reduce soil moisture via the pumping effect at the interface of the CO_2 gas plume and soil liquid water (Zhou et al., 2013) while also influencing the electrical conductivity of the soil (Zhou et al., 2012). The decreased ground cover at hot spots (and thus decreased LAI), contributes to changes in the soil moisture regime as shade is reduced and thus evapotranspiration is likely to increase (Zhou et al., 2012, 2013). Since soil pH (5 cm below the surface) was from 6.2 to 7.0, pH was not a likely additional stress on the plants at ZERT. Elevated CO_2 can influence nitrogen uptake at the species-specific level (Bassirad, 2000) and limit phosphorus content of roots (Newbery et al., 1995). When N and P are limited, anthocyanin production can increase (Close and Beadle, 2003), and cause the red-purple discoloration of leaves (Nittler and Kenny, 1976; Hodges and Nozzolillo, 1996). The nutrient status of dandelion and orchard grass leaves for N, P, and K indicates a sufficiency of these three nutrients (Kalra, 1998), but the sample size was limited due to a non-invasive experimental design. If sufficient nutrient levels are assumed, then the red-purple dandelion leaves at the ZERT site suggest that super-elevated soil CO_2 may have contributed to an increase in anthocyanin production. Anthocyanins function in protecting chlorophyll from photoinhibition (Sherwin and Farrant, 1998), scavenging H_2O_2 (Yamasaki et al., 1997), and protection of senescent leaves from photoinhibitory irradiances during nutrient reabsorption (Hoch et al., 2001), all of which may prove important during exposure of leaves to super-elevated CO_2 at the ZERT site.

Pigment change in orchard grass leaves was more subtle than in dandelion leaves. Orchard grass leaves changed from green to yellow, signifying a reduction in chlorophyll content (Adams et al., 1999), but only after three weeks of CO_2 injection in 2010 and during the second week of CO_2 injection in 2011. Chlorophyll was significantly reduced with super-elevated CO_2 at S_1 , in keeping

with previous research demonstrating that grass exposed to elevated soil CO₂ had shorter and browner leaves than in adjacent grasses (Pierce and Sjögersten, 2009) and that decreased chlorophyll content was due to an increase in soil CO₂ (Noomen and Skidmore, 2009). The slower and less pronounced pigment change in the C₃ orchard grass leaves at the ZERT site suggests that orchard grass leaves are more resistant than dandelion leaves to leaking CO₂. Both dandelion and orchard grass lost chlorophyll at the control site, but this was minimal and likely more closely related to the onset of seasonally normal leaf senescence than to leaking CO₂.

A decrease in chlorophyll is associated with an increase in accessory pigments such as the photooxidation-protective carotenoids (Peñuelas and Filella, 1998) when plants are under stress or senescing. For instance, Lakkaraju et al. (2010), used vegetation indices such as Ch1 NDI (chlorophyll normalized difference vegetation index), PSSRa and PSSRb (pigment specific simple ratios), and SIPI (structural independent pigment index) to demonstrate that a decrease in chlorophyll content was associated with an increase in carotenoid pigments at the hotspot. *Nardus stricta* L. had an approximately 33% decrease in chlorophyll content along with reduced RuBisCO content when growing near a cold, naturally emitting CO₂ spring in Iceland, possibly due to a decreased investment in the biochemical components of the photosynthetic apparatus (Cook et al., 1998).

Stomata have a high sensitivity to atmospheric CO₂ (Talbot et al., 2006) and stomatal conductance is an important factor in CO₂ uptake and in water loss via transpiration. Mid-day stomatal conductance increased in dandelion and orchard grass leaves with super-elevated soil CO₂ levels at S₁ (up to 18% by volume) and S₂ in 2010 and at S₀ and S₁ in 2011. Stomata respond to intercellular CO₂ concentrations rather than CO₂ concentrations either at the stomatal pore or the leaf surface (Mott, 1998). Potassium, malate and chloride increase in guard cells when stomata open and decrease when they close. Stomatal opening is associated with K⁺ uptake and closing is associated with a decrease in sucrose content (Talbot and Zeiger, 1998). With elevated atmospheric CO₂, guard cell membrane potential has greater depolarization, resulting in decreased stomatal aperture and closing of the stomata (Ainsworth and Rogers, 2007). However, Wheeler et al. (1999) showed that with very high or super elevated atmospheric CO₂ (5000–10,000 μmol mol⁻¹) stomatal conductance was greater at 5000–10,000 μmol mol⁻¹ CO₂ than at 1000 μmol mol⁻¹ in potato, soybean and sweet potato.

Stomatal conductance in soybeans decreased when atmospheric CO₂ increased from 400 to 1200 μmol mol⁻¹, but conductance increased with an increase from 1200 to 10,000 μmol mol⁻¹ CO₂, and these super-elevated concentrations of CO₂ induced a greater stomatal opening at mid-day and reduced stomatal closure at night (Levine et al., 2008). With water stress, abscisic acid (ABA) mediates reduction of transpiration rate by closing the stomata (Zhang and Davies, 1989). Increasing CO₂ to 10,000 μmol mol⁻¹ resulted in failure of ABA-mediated stomatal closure (Levine et al., 2008) with the net result of greater stomatal opening and changes in drought tolerance and water use efficiency. The greater stomatal conductance in dandelion leaves at the ZERT hotspot suggests that they suffered greater transpirational water losses than with closed stomata, resulting in leaf desiccation and dieback, and it is consistent with previous studies where mid-day stomatal conductance increased at super-elevated atmospheric CO₂ (10,000 μmol mol⁻¹), (Wheeler et al., 1999; Levine et al., 2008). Increased stomatal conductance may provide a partial explanation for leaf dieback and the development of the hotspots at the ZERT site.

Stomatal conductance was higher in orchard grass leaves than in dandelion leaves at all measurement dates and at all positions, possibly due to their species-specific physiological and anatomical characteristics. Although both species are amphistomatic,

dandelions leaves are mesophytic with larger surface areas, while grasses are generally more xeromorphic with less surface area. While leaf surface area and xeromorphy are important aspects of species-specific physiology and anatomy which can influence their responses to elevated CO₂, another important factor is stomatal density. With high atmospheric CO₂, stomatal density declined in some species (Woodward et al., 2002), remained the same (Kelly et al., 1991), and increased in others (Levine et al., 2008).

Increased fluorescence is an indicator of stress in plants, since plants under stress have proportionately more photons emitted as fluorescence and fewer traveling through the photochemical reactions of photosynthesis (DeEll et al., 1995; Hamerlynck et al., 2000; Rai et al., 2011). F_v/F_m represents the maximum efficiency of PSII photochemistry and is calculated from $F_m - F_o/F_m$ where F_m is maximum fluorescence and F_o is minimal fluorescence. Under non-stressful conditions, F_v/F_m has a red spectral peak at approximately 680 nm, with an optimal F_v/F_m ratio of 0.79 to 0.84 (Maxwell and Johnson, 2000). With stress, increased fluorescence results in a decreased F_v/F_m ratio (Gallé and Flexas, 2010). In dandelion leaves at the ZERT site, the average F_v/F_m ratios were below 0.79 at all stations along the transect, indicating a generally stressed population of dandelions in the plant study area. F_v/F_m ratios close to the hot spot were not significantly lower than those found at S₄, the control site. Therefore, dandelion leaves at the ZERT site did not appear to have significantly impaired PSII activity in response to super-elevated soil CO₂. However, these dandelions may also have been subjected to water stress invoked by the late summer drought at the ZERT site. Elevated CO₂ can lead to pH changes within plant cells, although cellular pH is generally well-regulated unless changes in CO₂ concentration are very large (Smith and Raven, 1979; Bown, 1985; Macek et al., 2005), as in the example of cytosolic pH that decreased from 6.9 in the control to 6.4 in avocado fruit at 40% CO₂ (Lange and Kader, 1997). Therefore, super-elevated CO₂ may have induced concurrent pH stresses in plants at the ZERT site.

Responses to elevated CO₂ are species-specific (Davey et al., 2006). For example, under elevated atmospheric CO₂, a hybrid poplar, *Populus × euramericana* Dode (Guinier) (*Populus deltoides* Bart. ex Marsh. × *Populus nigra* L.) exported more than 90% of its photosynthate and temporarily stored it in a large sink capacity (Davey et al., 2006). Conversely, *Lolium perenne* L. exposed to elevated CO₂ had a decreased sink capacity (Rogers and Ainsworth, 2006) due to insufficient nitrogen (Fischer et al., 2008; Isopp et al., 2001; Ainsworth and Rogers, 2007). Species-specific differences in the kinetics of nutrient uptake with high CO₂ may influence competition and thus community composition (Bassirirad, 2000).

At the ZERT hot spots, dandelions were more scarce than orchard grass following exposure to super-elevated soil CO₂, suggesting a greater tolerance of orchard grass to super-elevated soil CO₂, and potential domination of orchard grass over dandelion at the hot spots. Krüger et al. (2009), found that communities exposed to the elevated soil CO₂ of a mofette shifted toward a greater abundance of plants tolerant of more anaerobic or hypoxic conditions. Responses of plants to super-elevated soil CO₂ at mofettes are well documented (Kaligarić, 2001; Macek et al., 2005; Pfanz et al., 2005; Vodnik et al., 2005) and provide the perspective of plant responses to elevated CO₂ over many generations (Raschi et al., 1997; Onoda et al., 2007). Seeds of the grass, *Echinochloa crus-galli* (L.) P. Beauv., germinated under the highest CO₂ concentrations at a Slovenian mofette, where the vegetation pattern changed with increasing CO₂ and *Juncus effusus* L. and *Polygonum aviculare* L. became abundant (Kaligarić, 2001). Thus, changes in species distributions in response to super-elevated CO₂ at the ZERT site have many parallels in terms of plant responses to naturally occurring super-elevated CO₂ at mofettes.

5. Conclusions

The ZERT experiments on surface detection of leaks from CO₂ sequestration fields were also designed for research on the environmental effects of super-elevated soil CO₂. Specifically, we investigated the effects of super-elevated soil CO₂ for two consecutive years on *T. officinale* (dandelion) and *D. glomerata* (orchard grass), codominant species within the plant study area at ZERT and common field plants of lands where CO₂ sequestration fields might be placed. Investigation of these two plants provided insights into the responses of individual species to super-elevated soil CO₂, which, can then be placed in an environmental context.

Hot spots at the ZERT site formed in response to upwelling, super-elevated soil CO₂. These circular zones of leaf dieback appeared in consecutive years at the ZERT site and appeared markedly different from the surrounding vegetation that was not exposed to upwelling CO₂. Dandelion and orchard grass at the ZERT site responded to super-elevated soil CO₂ with a variety of visible stress responses that included color change from green to reddish purple, desiccation, and accelerated leaf dieback. Other physiological responses included decreased chlorophyll content and increased stomatal conductance. Further studies could examine the question of whether visible stress responses can be detected and compared with normal, seasonal changes in plant leaf pigmentation and other physiological attributes. The results of these future studies could be useful in implementing visible stress responses as tools in surface detection for year-round monitoring. Therefore, these and other physiological responses of plants to CO₂ leakage are useful indicators of leaking CO₂ from carbon sequestration sites in vegetation growing above sequestration fields and at naturally occurring CO₂ vents.

It is important to note that the visible stress responses of dandelion and orchard grass leaves to upwelling CO₂ occurred with exposure to super-elevated soil CO₂. This has not been studied as extensively as have plant responses to elevated atmospheric CO₂ that have been investigated at FACE facilities and other experiments where CO₂ levels are experimentally elevated to the levels of 500–100 ppm predicted with climate change (IPCC, 2007). However, CO₂ springs, or mofettes, offer insights into the responses of plants at naturally occurring sites of super-elevated soil CO₂, where Macek et al. (2005), found decreased rates of root respiration in species of grass, including *D. glomerata*, a species that is physiologically capable of growing naturally at both a natural spring in Slovenia and at the ZERT site.

In summary, dandelion and orchard grass provided a signature of visible stress responses that proved valuable in surface detection of leaks from the experimental CO₂ sequestration field at the ZERT facility, where circular hot spots of leaf dieback formed above leaks of CO₂. Within the hot spots, leaf physiology was disrupted as was evident by increased stomatal conduction, decreased chlorophyll content and rapid leaf dieback. Dandelion leaves were more sensitive to super-elevated soil CO₂ than orchard grass leaves. These stress responses were visible very quickly and noticeably following exposure to super-elevated soil CO₂ and are thus useful indicators of leaking CO₂.

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