Measurement and Partitioning of $In\ situ\ CO_2$ Fluxes in Turfgrasses Using a Pressurized Chamber

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ABSTRACT

Field measurements of photosynthesis in turfgrass often are conducted with handheld chambers that are temporarily placed over the canopy and soil. Because gas exchange measurements include soil respiration (R_s) , results do not represent net canopy photosynthesis $(P_{c,net})$ but rather net ecosystem exchange of CO_2 (NEE), or gross canopy photosynthesis (P_g) less the sum of canopy respiration (R_c) and R_s . Chambers attached to steady-state, portable photosynthesis systems normally are partially pressurized which may partially suppress $R_{\rm s}$ (depending on pressure magnitude and soil moisture and type) and may overestimate NEE. Objectives of this research were to: 1) develop a chamber in which pressure could be manipulated; 2) measure CO₂ fluxes at neutral pressure (chamber pressure equals atmospheric) to estimate NEE; 3) measure $P_{c,net}$ by applying increasing pressure until R_s is prevented from entering the chamber; and 4) partition CO_2 fluxes among P_g , R_c , and R_s . Pressure fluctuations of ±1.0 Pa were uncontrollable which caused large differences in CO₂ exchanges near neutral pressure and prevented measurements of NEE. Chamber pressurization from 50 to 200 Pa suppressed most but not all R_s which disallowed measurement of P_{cnet} using a single pressurized reading. A new procedure provided estimates of $P_{c,net}$, R_c , and P_g from pressurized measurements of turf under sunlit and shaded conditions and from turf clipped at ground level. Flux from clipped plots under pressurization provided estimates of R_s that could not be suppressed with pressure. This was subtracted from pressurized readings over sunlit and shaded unclipped canopies to estimate $P_{c,net}$ and R_c , respectively.

Key Words: Photosynthesis; surface chamber; turfgrass carbon balance; chamber pressure effects;

Abbreviations: LAI, leaf area index; *NEE*, net ecosystem exchange of CO_2 ; $P_{c,net}$, net canopy photosynthesis; P_g , gross photosynthesis; R_c , canopy respiration; R_s , soil respiration; R_s , residual soil respiration at high chamber pressures.

Photosynthesis is fundamental to plant function and can be a good indicator of plant stress and growth (Salisbury and Ross, 1978; Farquhar and Sharkey, 1994). In turfgrass studies, the effects of various stresses (e.g., drought, heat, cold) on turf health are important in determining turfgrass suitability for certain environments such as in various geographical regions or in golf courses, sports fields, etc. (Mancino, 1993; Qian et al., 1997; Huang et al., 1997). Small custom surface chambers that are attached to steady-state, portable photosynthesis systems and that cover a small portion of the turf's canopy are becoming increasingly popular to measure canopy photosynthesis in the field (Huang et al., 1998; Huang and Gao, 1999; Jiang and Huang, 2000; Xu and Huang, 2000). However, results from these measurements include the various components of the turf's ecosystem carbon (C) balance including photosynthesis, canopy respiration and soil respiration and thus, do not explicitly represent canopy photosynthesis.

The instantaneous CO₂ balance in turfgrass can be represented by:

$$NEE = P_{g} - R_{c} - R_{s}$$
 [1]

where *NEE* is net ecosystem exchange of CO₂, P_g is gross photosynthesis, R_c is canopy respiration, and R_s is soil respiration, all in μ mol m⁻² s⁻¹. Thus, measurements with the surface chamber placed over the top of turfgrass actually represent *NEE* rather than net canopy photosynthesis ($P_{c,net}$; μ mol m⁻² s⁻¹):

$$P_{\text{c.net}} = P_{\text{g}} - R_{\text{c}}$$
 [2]

Consequently, differences among treatments from chamber measurements of CO_2 flux in turfgrass include differences in R_s as well as in $P_{c,net}$. Note: In this manuscript, positive values are used for all variables in the carbon balance (i.e., P_g , R_c , and R_s ; Eqs. 1 and 2).

Soil respiration is sensitive to changes in chamber pressure (Kanemasu et al., 1974; Owensby et. al, 1997; Fang and Moncrieff, 1998; Lund et al., 1999) which may further confound results from the chamber. For example, positive pressure differences of a few tenths of a Pascal between the chamber interior and atmospheric (i.e., pressurization) may partially suppress R_s whereas negative pressure differences of as little as -1 Pa (i.e., suction) may cause an increase in CO_2 flux measurements by an order of magnitude (Fang and Moncrieff, 1998). When portable photosynthesis systems like the Licor 6400 (Li-cor, Lincoln, NE) are adapted for turfgrass studies (Huang et al., 1998; Huang and Gao, 1999; Jiang and Huang, 2000; Xu and Huang, 2000), the chamber is pressurized slightly above atmospheric although the exact amount of pressurization is usually not known. Because the amount of R_s suppression may vary with chamber pressure and soil conditions, the R_s suppressed by the chamber is also unknown. Therefore, measurements with the surface chambers may partially suppress R_s and overestimate NEE. Throughout this manuscript, 'chamber pressure' (negative or positive) refers to differential pressures between the chamber interior and outside the chamber (i.e., atmospheric).

The sensitivity of R_s to chamber pressure is affected by factors such as soil water content and soil properties (Lund et al., 1999). In general, dry and coarse soils show the most sensitivity to chamber pressure anomalies because of their greater soil permeabilities. Therefore, in drought studies in turfgrass in which plots may differ significantly in soil water content, a greater percentage of R_s may be suppressed in dry plots than in wet plots. Similarly, if comparisons are made among plots with differences in soil types, a greater percentage of R_s may be suppressed in coarser soils.

Because of the sensitivity of R_s measurements to chamber pressure, and because chamber pressure typically is not known in an open-flow system, CO_2 flux measurements may be suspect because they do not necessarily represent either *NEE* or $P_{c,net}$ (Eqs. 1 and 2). Observed differences in chamber measurements among treatments may be more related to differences in R_s

than to differences in $P_{c,net}$. Research is needed to develop a chamber measurement protocol that accounts for the effects of pressure on R_s in turfgrass studies. In this project, we propose that the chamber can be intentionally pressurized to almost completely suppress R_s and obtain independent measurements of P_g and R_c .

The objectives of this research were to: 1) fabricate and test a chamber connected to a portable photosynthesis system in which chamber pressure could be manipulated; 2) measure CO_2 fluxes at neutral pressure to obtain estimates of NEE; 3) apply increasing pressure until R_s is prevented from entering the chamber; and 4) attempt to partition the various components in the turf C balance, including P_g , R_c , and R_s in three cool-season turfgrasses. The result would be a more accurate indicator of treatment effects on $P_{c,net}$

Theory

The *NEE* (Eq. 1) of turfgrass could be determined by measuring CO₂ fluxes with the chamber at neutral pressure (i.e., the pressure inside the chamber is equal to the pressure outside). Because the chamber in steady-state (i.e., open) systems normally has positive pressure, the airstream would need to be exhausted or pumped from the chamber at the same rate that it enters. This would result in better equilibrium between the chamber and atmospheric pressure.

Because it is known that positive chamber pressures suppress R_s , we hypothesized that R_s could be completely suppressed from chamber measurements if the pressure was purposely increased sufficiently. Suppressing R_s from chamber measurements would eliminate R_s from Eq. 1 and thus, would yield $P_{c,net}$ (Eq. 2). Furthermore, R_s could be estimated by the difference between neutral-pressure (Eq. 1) and over-pressure (Eq. 2) measurements.

Covering the chamber with an opaque container during overpressurized measurements would result in R_c separately, assuming: 1) R_c was not affected by significantly affected by covering (i.e., sudden darkness); 2) photosynthesis (P_g) could be sufficiently eliminated by covering; and 3) R_s was eliminated by pressure (Eq. 1). Thus, sunlit and shaded measurements under pressurization on the same area would yield $P_{c,net}$ and R_c , respectively. From Eq. 2 it is evident that P_g can be estimated from the sum of the sunlit ($P_{c,net}$) and shaded (R_c) readings. This strategy for partitioning the CO₂ balance is summarized in Table 1.

From the method just described we could estimate $P_{\rm g}$, $R_{\rm c}$, and $R_{\rm s}$ separately, which are more directly related to the biophysics of the canopy and would provide significantly more information about the effects of various stresses on the individual components of the turf C balance. This information would also be of use to modelers who may be interested in predicting the effects of various environmental stresses on the individual components in turfgrass C balances.

MATERIALS AND METHODS

The study was conducted from mid July to October 2003 at the Rocky Ford Turfgrass Research Center and Ashland Bottoms, both near Manhattan, Kansas (39.12°N, 96.35°W). The soil at Rocky Ford was Chase silt loam (fine, montmorillonitic, mesic, Aquic Arquidolls) and at Ashland Bottoms was Sarpy loamy fine sand (mixed, mesic, Typic Udipsamments). Gas exchange measurements were collected from bare soils and from established stands of three cool-season turfgrasses: perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Screb.), and Kentucky bluegrass (*Poa pratensis* L.). Turfgrasses were mowed once to twice weekly at 6.35 cm.

Measurements of gas exchange

Whole canopy gas exchange measurements were collected with a portable photosynthesis system (LI-6400, LICOR, Inc., Lincoln, NE) equipped with a 0.64-L custom chamber that covered a surface area of 7.09 x 10⁻³ m² (i.e., 9.5 x 10⁻² m diam.). The custom chamber was constructed from Plexiglass and the chamber interior was lined with Teflon tape to improve water sorption and desorption properties of the chamber walls. A thin aluminum edge (2 cm deep) was attached along the base of the chamber to push into the soil and improve the seal between the chamber and surface. The chamber was modified to allow for adjustment and measurement of chamber pressure (Fig. 1). Three ports were installed on the custom chamber. Flow rate and hence, chamber pressure was adjusted by pumping air either into or out of the chamber through one port by using an adjustable air pump (UNMP30, KNF Neuberger, Inc., Trenton, NJ). A second port was used to measure chamber pressure using a digital micromanometer (DMI, Infeltec, Waynesboro, VA) with a resolution of 0.1 Pa. The third port had an adjustable valve installed to restrict airflow out of the chamber. By restricting airflow out of the chamber, higher chamber pressures could be achieved provided a tight seal was achieved between the chamber base and collar.

Flow rate into the chamber from the KNF pump was measured with a mass flowmeter (GFM17, Aalborg, Orangeburg, NY). When additional airflow was introduced into the chamber to create higher pressures it diluted CO₂ concentrations in the chamber and thus, it was necessary to modify the gas exchange calculations. The modified mass balance of CO₂ in the open system was:

$$a = \frac{u_{i1}(c_i - c_o) + u_{i2}(c_i - c_o)}{s} - Ec_o$$
 [3]

where a is net assimilation rate (mol CO₂ m⁻² s⁻¹), u_{i1} is the normal incoming flow rate from the open system (mol air s⁻¹), u_{i2} is the added incoming flow rate from the variable rate pump (mol air s⁻¹), c_i is the incoming ambient CO₂ concentration (mol CO₂ mol air⁻¹), c_o is the outgoing CO₂ concentration (mol CO₂ mol air⁻¹), s is surface area covered by the chamber (m²), and E is evaporation from the leaves and soil surface (mol H₂O m⁻² s⁻¹). The modified mass balance of water vapor in the open system was:

$$E = \frac{u_{i1}(w_o - w_i) + u_{i2}(w_o - w_i)}{s(1 - w_0)}$$
 [4]

where w_o is the outgoing H₂O mole fraction and w_i is the incoming H₂O mole fraction, both in mol H₂O mol air⁻¹. Raw output from the Li-cor 6400 was transferred to a PC and the corrections were made on a spreadsheet.

Chamber measurements of CO_2 flux were collected from well-watered, intact turf canopies under full sunlight and when shaded with an opaque covering. Measurements of R_s also were collected from bare soil surrounded by perennial ryegrass, big bluestem (*Andropogon gerardii*), and in an open sandy area devoid of vegetation. Poly-vinyl chloride (PVC) collars (9.5 cm diam. x 8 cm) were driven 6 cm into the ground at each measurement site at least 24 hours before gas exchange measurements were collected. The 24-h delay allowed for dissipation of any soil CO_2 that may have been released by disturbance of wet soils during installation of the collars (Norman et al., 1992). A foam gasket was constructed to improve the seal between the chamber base and the collars. Measurements were collected first at neutral chamber pressure by withdrawing air with the KNF pump. Then by closing the shut-off valve on the exhaust line and adding air using the KNF pump, data were collected at sequentially higher pressures until R_s was

suppressed; measurements were collected sequentially under full sunlight and shaded conditions, respectively, at each increasing pressure increment.

Green leaf area index, aboveground biomass, and soil water content

On October 14, 2003, green leaf area index (LAI) and aboveground biomass were harvested and measured from each collar immediately after gas exchange measurements were collected. Green LAI was measured with an area meter (LI-3100, Li-Cor, Lincoln, NE), and total aboveground biomass was determined gravimetrically after samples had been dried in a forcedair oven for 48 h at 60°C. Gravimetric soil water content data were collected from 0-10 cm at each site whenever gas exchange measurements were collected.

RESULTS AND DISCUSSION

Measurements at neutral pressure

Maintaining neutral chamber pressure using the manometer and variable rate air pump was extremely difficult in the field. Windy conditions, which were nearly always present during midday measurements of gas exchange, caused uncontrollable fluctuations in manometer readings of ± 1.0 Pa and caused difficulty in controlling chamber pressure. Unfortunately, uncertainties of ± 1.0 Pa in chamber pressure caused large differences in CO₂ exchange near neutral pressure ("zero" Pa; Fig. 2), where either slight suction or pressure can cause significant changes in R_s entering the chamber (Kanemasu et al., 1974; Owensby et. al, 1997; Fang and Moncrieff, 1998; Lund et al., 1999). Consequently, we could not be certain whether chamber pressure was precisely neutral.

Erratic measurements of CO_2 exchange near neutral pressure illustrated the effect of uncertain chamber pressures to within ± 1.0 Pa as measured with the manometer (Fig. 2). For example, the sum of R_c and R_s at "zero" Pa in a shaded ryegrass canopy was 44.5 μ mol m⁻² s⁻¹ (Fig. 2B), which was unrealistically large and was likely inflated by an actual slight negative pressure (i.e., suction) inside the chamber (Fang and Moncrieff, 1998). Conversely, the apparent *NEE* in the sunlit canopy were greater at 0 Pa (8.60 μ mol m⁻² s⁻¹) than at 0.5 Pa (3.27 μ mol m⁻² s⁻¹; Fig. 2A), indicating more R_s was suppressed at neutral pressure than at 0.5 Pa. However, the chamber pressure differential at the neutral reading may have actually been greater than 0 Pa or even greater than 0.5 Pa which may have suppressed R_s and inflated apparent NEE.

Measurements of R_s also were erratic between 0 and 1 Pa (Fig. 2C). Because of the problems associated with maintaining neutral chamber pressures we were unable to collect measurements of *NEE* in this study. This was a setback in our attempt to partition the CO_2 balances as described in Table 1. However, overpressurization to suppress R_s in the chamber could still potentially lead to estimates of P_g and R_c .

Pressure chamber measurements

Data collected on bare soil showed that R_s was substantially suppressed as chamber pressure increased (Figs. 2C and 3). For example, R_s in silt loam decreased from 14.35 μ mol m⁻² s⁻¹ at low chamber pressures to 1.58 μ mol m⁻² s⁻¹ at 50 Pa (Fig. 3A). Likewise, R_s was suppressed in sand as chamber pressure increased although the fluxes were initially smaller in sand than in silt loam (Figs. 3B and 3C). Smaller R_s in sandy soils was likely caused by less organic matter and drier conditions; gravimetric soil water content was 14% in the silt loam and 8-10% in the sands. In Figure 2C, the suppression of R_s (bare soil) with increasing chamber pressure caused

the corresponding increase in net CO₂ uptake during the sunlit reading (Fig. 2A) and the decrease in CO₂ emissions during the shaded reading (Fig. 2B).

Manometer readings indicated that under normal operation (i.e., without intentionally pressurizing the chamber), pressure in the chamber ranged from 7 to 78 Pa. Therefore, the amount of R_s suppressed during normal operation probably varied spatially and temporally because of differences in chamber pressure (Figs. 2C and 3). Lower chamber pressures under normal operation would allow more R_s into the chamber and thus, reduce "canopy photosynthesis" measurements compared to higher pressures where R_s was prevented from entering the chamber. Comparisons between chamber measurements under normal operation and when overpressurized confirmed that R_s was typically only partially suppressed under normal operation (Fig. 4). For example, CO_2 exchange measurements with the chamber under normal operation ranged from 6 to 23% lower than when the chamber was overpressurized.

Measurements in Figure 4 were collected from perennial ryegrass, tall fescue, and Kentucky bluegrass when gravimetric soil water content in these turfgrasses ranged from 22 to 29%.

Although R_s could be significantly reduced with intentional chamber pressurization, R_s could not be completely eliminated (Figs. 2C and 3). In a wet silt loam soil (26% gravimetric soil water content), R_s remained at 1.92 µmol m⁻² s⁻¹ despite chamber pressures as great as 714 Pa (Fig. 2C); between 100 and 714 Pa, only an additional 0.36 µmol m⁻² s⁻¹ was suppressed. In another silt loam soil with 14% gravimetric soil water content, R_s remained at 1.58 µmol m⁻² s⁻¹ at the highest pressure that could be maintained at that location (50 Pa; Fig. 3A). Although a greater amount of R_s was suppressed in drier sands than in silt loams, R_s still could not be completely eliminated and remained at 0.34 to 0.64 µmol m⁻² s⁻¹ (Figs. 3B and 3C).

Recommended protocol for pressurized measurements

Because not all R_s could be eliminated from chamber measurements, an alternative procedure was developed to account for this small soil flux that could not be suppressed and yet allow for estimates of $P_{c,net}$, R_c , and P_g . This was accomplished by measuring CO_2 fluxes with an overpressurized chamber in sunlit and shaded canopies and then taking another pressurized reading after clipping the turf canopy at ground level from an area in or near the plots. This provided an estimate of the R_s , the soil flux entering the chamber when it was overpressurized. Thus, three measurements (i.e., from sunlit, shaded, and clipped canopies) with an overpressurized chamber provided estimates of the following components of the C balance:

Sunlit chamber =
$$P_g$$
- R_c - R_s ' [5]

Shaded chamber =
$$R_c$$
- R_s ' [6]

Clipped canopy=
$$R_s$$
' [7]

From Eq. 5-7, estimates of $P_{c,net}$, P_{g} , and R_{c} could be calculated:

$$P_{\rm g} = Sunlit\ chamber - shaded\ chamber$$
 [8]

$$P_{c,net} = Sunlit \ chamber - clipped \ canopy$$
 [9]

$$R_{\rm c} = Shaded\ chamber - clipped\ canopy$$
 [10]

Research by others has indicated that dark respiration in leaves is inhibited from 17 to 77% by light in a number of agricultural and woody species (Villar et al., 1994; Villar et al., 1995; Atkins et al., 1997), suggesting that R_c may increase after the canopy is covered. Atkins et al. (1997) found that in seven grass (Poa) species, dark respiration was inhibited an average of 30-35% by light although 25 to 30 min were required following the onset of darkness before respiration rates stabilized. In the present study we assumed that R_c did not change significantly in the 3 to 4 min that were required to complete each chamber measurement immediately

following covering. Further research may be required to measure the rate of change of R_c following covering and to determine its effect on estimates of P_g as described above.

Overpressurization was achieved by closing the valve in the chamber's exhaust line and pumping additional air into the chamber at a rate of about 700 µmol s⁻¹ (~1.0 L min⁻¹). The chamber pressure when overpressurized varied considerably with location. For example, chamber pressure on a bare silt loam was 714 Pa while the maximum pressure over a nearby ryegrass canopy was only 200 Pa (Fig. 2). Presumably chamber pressure may vary with soil type and soil moisture. However, the tightness of the seal between the chamber base and the collar may have been equally important in maintaining higher pressures. A foam gasket was constructed to improve the seal and in general, resulted in higher chamber pressures than when no gasket was used. Leaks in the seal between the chamber base and collar were easily detected with the manometer through a rapid drop in chamber pressure. The seal could generally be improved by repositioning the chamber and gasket on the collar.

When using this technique, field measurements suggested that chamber pressures of 50 Pa should be maintained where possible and no less than 20 Pa. For example, measurements over bare soils indicated that 83 to 94% of R_s was suppressed at chamber pressures of 50 Pa or greater, while 76 to 89% of R_s was suppressed at 20 Pa. Below chamber pressures of 20 Pa the amount of R_s suppressed declined rapidly (Figs. 2C and 3). The same chamber pressure must be maintained during measurements over the unclipped and clipped plots.

Measurements from clipped areas with the overpressurized chamber (i.e., R_s ') ranged from 2.13 µmol m⁻² s⁻¹ in Kentucky bluegrass to 4.71 µmol m⁻² s⁻¹ in tall fescue (Table 2), which was slightly greater than pressurized measurements from nearby bare soil (Figs. 2C and 3). Despite careful clipping near the ground, it was not possible to remove crowns of the turfgrass

plants; crowns are regions of high meristematic activity. Thus, measurements in clipped areas likely contained crown respiration as well as the small amount of soil CO_2 flux entering the pressurized chamber. Therefore, crown respiration was a larger fraction of R_s ' than R_c ; R_c was primarily respiration from green leaves. Clipping injury also may have affected estimates of R_s ' although little data are available in the literature regarding the temporal responses of grass stem or crown respiration immediately following clipping injury. In our study, chamber measurements were taken over the clipped area within two to three minutes after clipping, which presumably precluded any changes in R_s ' that may have evolved from clipping injury.

Estimates of $P_{c,net}$ ranged from 11.54 to 14.33 µmol m⁻² s⁻¹ and P_g from 14.07 to 17.37 µmol m⁻² s⁻¹ (Table 2). Estimates of $P_{c,net}$, R_c , and P_g were greater per unit ground area in perennial ryegrass and tall fescue than in Kentucky bluegrass. Interestingly, when data were normalized for LAI, $P_{c,net}$, P_g , and R_c were similar among all species. Leaf area index was 1.91 m² m⁻² in perennial ryegrass, 2.06 m² m⁻² in tall fescue, and 1.72 m² m⁻² in Kentucky bluegrass; and aboveground biomass was 3.09 g m² in perennial ryegrass, 3.79 g m² in tall fescue, and 2.52 g m² in Kentucky bluegrass.

In summary, partitioning of C fluxes provided more sensitive and meaningful comparisons between treatments than did measurements with the unmodified chamber because of the direct relationship of P_g , $P_{c,net}$, and R_c to the biophysics of the turfgrass canopy. Further research and development are needed to construct a chamber that can be operated in both pressurized and neutral-pressure modes to allow for the partitioning of NEE and R_s . Although testing was done on turfgrass, the principles could theoretically be used on any low-growing vegetation.

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Table 1. Proposed strategy for partitioning the CO_2 balance in turfgrasses using measurements from a small surface chamber modified to manipulate chamber pressure. Individual components include net ecosystem exchange (NEE) of CO_2 , gross canopy photosynthesis (P_g), canopy respiration (R_c), soil respiration (R_s), and net canopy photosynthesis ($P_{c,net}$).

Pressure	Radiation	Flux Measured			
Neutral	Sunlit	$NEE = P_{\rm g} - R_{\rm c} - R_{\rm s}$			
Overpressure	Sunlit	$P_{\mathrm{c,net}} = P_{\mathrm{g}} - R_{\mathrm{c}}$			
Overpressure	Shaded	$R_{ m c}$			
By calc	$R_{\rm s} = NEE - P_{\rm c,net}$				
By calc	$P_{ m g} = P_{ m c,net} + R_{ m c}$				

Table 2. Measurements of CO_2 fluxes in turfgrasses with the overpressurized, modified chamber and subsequent estimates of net canopy photosynthesis ($P_{c,net}$), canopy respiration (R_c), and gross photosynthesis (P_g). Measurements were collected in perennial ryegrass (PR), tall fescue (TF), and Kentucky bluegrass (KBG) on October 14, 2003.

5	Chamber Measurements					Carbon Balance Components					
6		Canopy Conditions			Per	Per Unit Ground Area			Normalized for Leaf Area		
7	Species	Sunlit	Shaded	Clipped	$P_{c,net}$	<u> </u>		$P_{ m c,net}$	<u> </u>	$P_{\rm g}$	
8		μmol m ⁻² gro			round s ⁻¹	ound s ⁻¹			μmol m ⁻² leaf s ⁻¹		
	PR	10.16	7.17	3.89	14.05	3.28	17.33	7.37	1.73	9.10	
	TF	9.62	7.75	4.71	14.33	3.04	17.37	6.94	1.48	8.42	
	KBG	9.41	4.66	2.13	11.54	2.53	14.07	6.70	1.47	8.17	
9											

- Figure 1. Conceptual diagram that illustrates the design of the pressurized chamber system. A
- 2 variable-flow air pump and micro-manometer were used to regulate chamber pressure. A
- mass flow meter was used to monitor the air flow into the chamber, and results were used to
- 4 correct fluxes for the effect of gas concentration dilution in the chamber.
- 5 Figure 2. Apparent values in perennial ryegrass of: A) net ecosystem exchange of CO₂ (*NEE*;
- sunlit chamber); and B) the sum of canopy and soil respiration ($R_c + R_s$; shaded chamber) in
- 7 response to changes in chamber pressure. The apparent increase in *NEE* and decrease in
- 8 R_c+R_s with chamber pressure were caused by the corresponding suppression of: (C) soil
- 9 respiration entering the chamber (R_s ; bare-soil); the bare soil area was surrounded closely by
- perennial ryegrass. Note: Scale of y-axis of 2B is different from 2A and 2C.
- Figure 3. Measurements of soil respiration as a function of increasing chamber pressure. Soils
- were bare silt loam surrounded by Kentucky bluegrass (A), sand between crowns of big
- bluestem (B), and sand in an area devoid of vegetation (C). Note: Scale of y-axis of 3A is
- different from 3B and 3C.
- 15 Figure 4. Comparison of CO₂ fluxes measured sequentially with the unmodified chamber
- 16 (normal operation; sunlit) and then with the modified chamber (pressurized; sunlit) in
- perennial ryegrass (PR), Kentucky bluegrass (KBG), and tall fescue (TF). PR and TF were
- measured on multiple dates.







