Increased C_3 productivity in Midwestern lawns since 1982 revealed by carbon isotopes in *Amanita thiersii*

Erik A. Hobbie[1] (iD), Brian A. Schubert[2] (iD), Joseph M. Craine[3] (iD), Ernst Linder[4] (iD), and Anne Pringle[5] (iD)

[1]Earth Systems Research Center, University of New Hampshire, Durham, New Hampshire, USA, [2]School of Geosciences, University of Louisiana at Lafayette, Lafayette, Louisiana, USA, [3]Jonah Ventures, Manhattan, Kansas, USA, [4]Department of Mathematics and Statistics, University of New Hampshire, Durham, New Hampshire, USA, [5]Departments of Botany and Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin, USA

Abstract How climate and rising carbon dioxide concentrations (pCO_2) have influenced competition between C_3 and C_4 plants over the last 50 years is a critical uncertainty in climate change research. Here we used carbon isotope ($\delta^{13}C$) values of the saprotrophic lawn fungus *Amanita thiersii* to integrate the signal of C_3 and C_4 carbon in samples collected between 1982 and 2009 from the Midwestern USA. We then calculated ^{13}C fractionation (Δ) to assess the balance between C_3 and C_4 photosynthesis as influenced by mean annual temperature (MAT), mean annual precipitation over a 30 year period (MAP-30), and pCO_2. Sporocarp Δ correlated negatively with MAT ($-1.74‰\,°C^{-1}$, 79% of variance) and positively with MAP ($9.52‰\,m^{-1}$, 15% of variance), reflecting the relative productivity of C_3 and C_4 grasses in lawns. In addition, Δ values correlated positively with pCO_2 ($0.072‰\,ppm^{-1}$, 5% of variance). Reduced photorespiration with rising pCO_2 accounted for 20% of this increased Δ, but the remaining 80% is consistent with increased assimilation of C_3-derived carbon by *Amanita thiersii* resulting from increased productivity of C_3 grasses with rising pCO_2. Between 1982 and 2009, pCO_2 rose by 46 ppm and the relative contribution of C_3 photosynthesis to *Amanita thiersii* carbon increased 18.5%. The $\delta^{13}C$ value of *Amanita thiersii* may integrate both lawn maintenance practices and the physiological responses of turf grasses to rising CO_2 concentrations.

1. Introduction

Fundamental differences between the C_3 and C_4 modes of photosynthesis account for the higher $^{13}C{:}^{12}C$ ratios (expressed as $\delta^{13}C$ values) observed in C_4 plants than in C_3 plants. These fundamental differences also explain the differential responses of C_4 and C_3 grasslands to changes in atmospheric carbon dioxide levels (pCO_2), temperature, precipitation, and nutrient availability. In mixed communities, the competitive balance between C_3 and C_4 grasses should accordingly be sensitive to climate change and ongoing increases in pCO_2. Researchers have used $\delta^{13}C$ values in grazers to examine the balance between C_3 and C_4 grasses in paleontological studies stretching back 5–8 Ma [*Cerling et al.*, 1997] and have done similar studies in modern grasslands [*Auerswald et al.*, 2012], although data are needed on responses to the sharply rising pCO_2 of the last 50 years [*Lattanzi*, 2010].

Grasses lack the long-term record of environmental responses that have been used so successfully in tree ring research. One approach to overcome this limitation used native prairie soils themselves as integrators of the productivity of C_3 versus C_4 plants [*von Fischer et al.*, 2008]; another promising approach is to use herbarium specimens as recorders of environmental information during the season of collection [*McLauchlan et al.*, 2010], although phylogenetic variability and the variable response to climate result in a noisy signal. Herbarium specimens of saprotrophic fungi may also provide a long-term record of environmental change since these fungi rely on plant cellulose as their primary source of energy and carbon.

One such fungus, *Amanita thiersii*, forms sporocarps in Midwestern lawns and is expanding its range rapidly [*Wolfe et al.*, 2012]. Lawns are the single largest irrigated crop in the U.S. and covered between 100,000 and 160,000 km^2 in 2005 (Figure 1) [*Steinberg*, 2006]. The $\delta^{13}C$ values of *Amanita thiersii* indicated that it can assimilate carbon derived from both C_3 and C_4 photosynthetic pathways [*Wolfe et al.*, 2012]. Here we will use isotopic patterns in *Amanita thiersii* to integrate local productivity of C_3 versus C_4 grasses and relate that productivity to climatic factors and pCO_2.
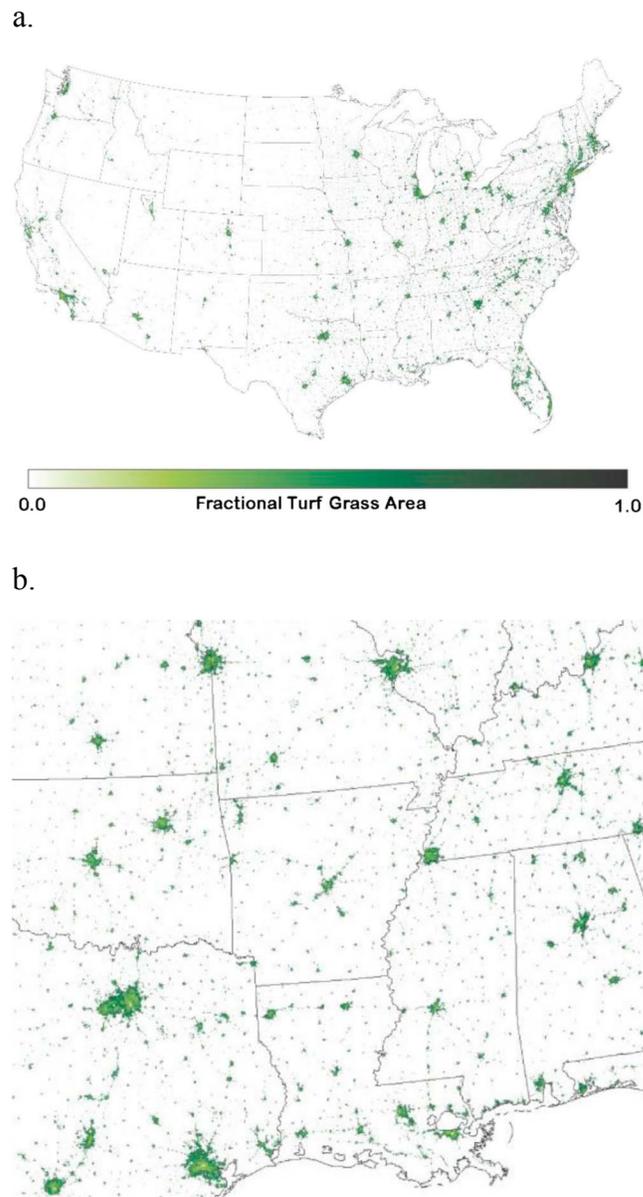
a.

b.

Figure 1. Fraction of USA surface area covered by lawns. From http://eoi-mages.gsfc.nasa.gov/images/imagerecords/6000/6019/frac_turf-grass_lrg.jpg. (a) United States. (b) Sampling area in south central USA.

Numerous factors can influence the balance of C_3 and C_4 photosynthesis at a site. Both temperature and precipitation have been linked to the relative proportions of C_3 and C_4 photosynthesis across large spatial scales [*Tieszen et al.*, 1997; *von Fischer et al.*, 2008]. However, agricultural maize production can also influence large-scale patterns of C_4 photosynthesis. The relative proportions of C_3 and C_4 photosynthesis were quantified spatially by *Still et al.* [2009] in a global data set of the relative proportions of C_4 photosynthesis (f_{C4}) at 1° resolution. Within the central United States the choice of lawn grasses depends on the climatic zone, with more C_3 grasses selected in cooler regions and more C_4 grasses selected in warmer regions [*Milesi et al.*, 2005]. Temperature is generally considered the dominant control on the abundance of C_3 versus C_4 grasses in natural habitats, with different studies using daytime summer maximum temperature, minimum growing season temperature, or mean annual temperature as the metric best correlating with the proportions of C_3 versus C_4 plants [*Ehleringer et al.*, 1997]. The proportion of annual precipitation falling in summer in natural grasslands is also positively correlated with C_4 abundance [*Paruelo and Lauenroth*, 1996].

The Suess effect, the decrease in $\delta^{13}C$ of atmospheric CO_2 caused by the burning of fossil fuels since the start of the Industrial Revolution, also influences $\delta^{13}C$ patterns. The $\delta^{13}C$ of atmospheric CO_2 has decreased by about 1.7‰ since 1850, while pCO_2 has risen from 285 ppm to ~400 ppm [*Hua et al.*, 2013; *McCarroll and Loader*, 2004]. One approach to remove the influence of the Suess effect is to calculate the ^{13}C discrimination (Δ) of the tissue in question relative to the $\delta^{13}C$ of atmospheric CO_2 [*Köhler et al.*, 2010]. This approach allowed *Schubert and Jahren* [2012] to show that ^{13}C discrimination increased in C_3 plants with increasing pCO_2 in elevated CO_2 experiments. However, long-term records of Δ from spring-harvested, seminatural grasslands at Rothamsted (all C_3 plants) indicated no shift in Δ from 1857 to 2007 [*Köhler et al.*, 2010].

Wolfe et al. [2012] compared $\delta^{13}C$ and $\delta^{15}N$ measurements on 49 archived specimens of *Amanita thiersii* against similar measurements from grassland *Hygrocybe* and from a worldwide survey of fungi to prove that *Amanita thiersii* was saprotrophic, rather than ectomycorrhizal. Here we have reanalyzed the isotopic data set of *Amanita thiersii* presented in *Wolfe et al.* [2012] against site information on mean annual temperature, mean annual precipitation, pCO_2, and the regionally estimated proportion of C_4 photosynthesis (f_{C4}) to
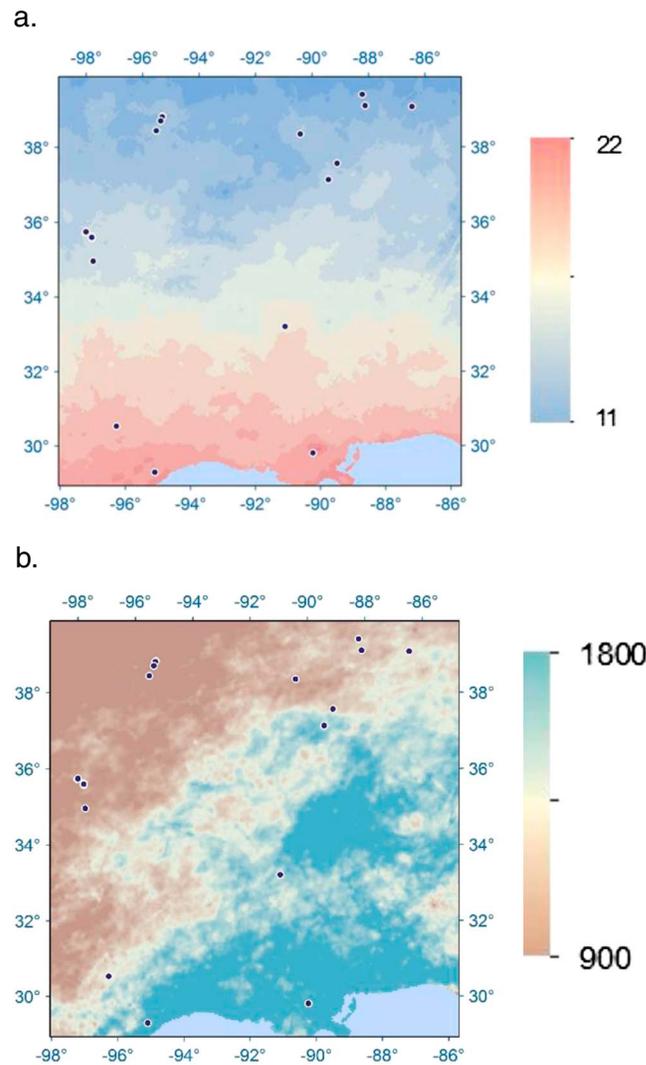
a.

b.

Figure 2. Collection locations are plotted by latitude (°N) and longitude (°W, given a negative sign) versus (a) mean annual temperature, MAT, and (b) mean annual precipitation, MAP. Colored isopleths are 1°C for temperature (from 11°C to 22°C) and 200 mm for precipitation (from 900 mm to 1800 mm). Isopleths for 2002 are shown.

study whether this fungus may integrate information on the competitive balance between C_3 versus C_4 grasses from the lawns where it was collected. We hypothesized the following:

1. $\delta^{13}C$ of *Amanita thiersii* integrates the relative proportions of C_3 and C_4 vegetation at the specific locations where it is collected; accordingly, Δ should correlate strongly with measures that either reflect or influence the balance of C_3 and C_4 vegetation such as f_{C4}, temperature, and precipitation.
2. Because pCO_2 has increased over time, Δ of *Amanita thiersii* will also increase over time [*Schubert and Jahren*, 2012].

2. Materials and Methods

In *Wolfe et al.* [2012], gill tissue was subsampled from 48 herbarium specimens of *Amanita thiersii* collected at 26 different locations between 1982 and 2009 in southeastern and south central USA. Locations were between 29°N and 40°N and 86°W and 100°W. Samples were analyzed for %C, %N, $\delta^{13}C$, and $\delta^{15}N$ as detailed in *Wolfe et al.* [2012]. We analyzed the underlying data set from *Wolfe et al.* [2012] (as provided by B. Wolfe) using multiple regressions as provided in the statistical software JMP (SAS Institute, Cary, North Carolina, USA). One outlier sample collected in 1952 was not used in the analyses.

Mean annual temperature (MAT) and mean annual precipitation (MAP) during the year of collection were determined with the Daymet Single Pixel Extraction tool using the latitude and longitude of sample sites (daymet.ornl.gov). In addition, mean annual temperature (MAT-30), mean annual precipitation (MAP-30), and mean temperature for the warmest month (WMT-30) and coldest month (CMT-30) for each location were derived from the 1961–1990 averages available from the Climate Research Unit (http://www.cru.uea.ac.uk), as described in *New et al.* [2002]. Yearly values of $\delta^{13}CO_2$ and pCO_2 were derived from *McCarroll and Loader* [2004], *Keeling et al.* [2009], *Hua et al.* [2013], and the publicly available database of the U.S. Earth Systems Research Laboratory (https://www.esrl.noaa.gov/gmd/ccgg/trends/data.html). From 1982 to 2009 the $\delta^{13}C$ value of atmospheric CO_2 declined from $-7.48‰$ to $-8.24‰$ because of the addition of fossil fuel-derived CO_2 to the atmosphere (the Suess effect). The Suess effect was quantified as the difference between the $\delta^{13}C$ value of atmospheric CO_2 in the year of collection and the preindustrial value of $-6.4‰$. We calculated the ^{13}C fractionation (Δ) from atmospheric CO_2 to *Amanita thiersii* as $\Delta = (\delta^{13}C_{CO2} - \delta^{13}C_{Amanita})/(1 + \delta^{13}C_{Amanita})$. To test if large-scale estimates of C_4 photosynthesis were useful predictors of the Δ values calculated for *Amanita thiersii*, we used C_4 photosynthesis estimates from *Still et al.* [2009] that gave the fraction of C_4 photosynthesis at 1° resolution ($f_{C4-1°}$). We also used the *Still et al.* [2009]

Table 1. Data on Annual pCO_2, the Suess Effect, Mean Annual Temperature (MAT), Mean Annual Precipitation (MAP), and $\delta^{13}C$[a]

Date (n)	pCO_2 (ppm)	Suess Effect (‰)	Lat (°N)	Long (°W)	MAT (°C)	MAT-30 (°C)	MAP (mm)	MAP-30 (mm)	$\delta^{13}C \pm se$ (‰)
11/9/1952	312.80	−0.46	30.61	96.36	20.57	20.0	836	991	−9.17
20/8/1982	341.13	−1.08	33.38	91.05	17.52	17.3	1345	1342	−14.00
15/7/1989	352.91	−1.28	35.66	97.47	15.64	15.6	938	851	−11.67
3/10/1990 (4)	354.19	−1.31	35.67	97.48	15.91	15.6	971	851	−10.67 ± 0.34
6/9/1991	355.59	−1.34	35.66	97.47	15.72	15.6	969	851	−13.57
8/9/1992 (2)	356.37	−1.37	37.73	89.21	13.57	13.2	122	1144	−20.83 ± 0.05
17/10/1999	368.31	−1.56	29.96	90.22	20.51	20.3	174	1589	−11.15
30/10/2000	369.48	−1.59	35.63	97.47	15.95	15.6	968	851	−12.56
7/6/2001	371.02	−1.62	35.63	97.47	15.89	15.6	951	851	−14.74
22/8/2001	371.02	−1.62	35.65	97.47	15.90	15.6	948	851	−11.97
15/9/2001	371.02	−1.62	35.02	97.38	16.33	16.0	1040	852	−14.08
2/9/2002	373.10	−1.65	35.78	97.70	15.85	15.7	926	816	−13.98
29/7/2003	375.64	−1.67	39.55	88.25	11.90	11.4	1072	994	−20.71
1/8/2003 (2)	375.64	−1.67	39.25	88.16	12.21	11.7	1087	1021	−21.29 ± 0.03
27/6/2004	377.38	−1.70	35.79	97.68	15.80	15.7	893	816	−11.18
28/6/2004 (2)	377.38	−1.70	38.53	90.45	13.42	13.1	1113	952	−21.49 ± 0.02
17/8/2004	377.38	−1.70	35.79	97.68	15.80	15.7	894	816	−10.69
18/7/2005 (2)	379.67	−1.73	37.30	89.52	14.18	14.1	1202	1184	−18.19 ± 0.33
4/7/2007 (4)	383.55	−1.78	38.97	95.25	13.17	13.3	988	992	−20.26 ± 0.37
22/7/2007	383.55	−1.78	39.55	88.25	12.22	11.4	1039	994	−22.08
10/9/2007	383.55	−1.78	39.16	86.52	12.02	11.7	1277	1095	−24.05
19/7/2008	385.34	−1.81	39.55	88.25	12.19	11.4	1086	994	−21.12
11/9/2008 (7)	385.34	−1.81	38.58	95.45	13.45	13.1	998	952	−19.98 ± 0.20
16/9/2008 (4)	385.34	−1.81	38.96	95.26	13.06	13.3	1000	992	−21.46 ± 0.16
22/7/2009 (4)	387.23	−1.83	38.85	95.31	13.07	13.3	976	992	−21.51 ± 0.14
29/8/2009 (2)	387.23	−1.83	29.41	95.12	21.02	20.5	1414	1138	−10.22 ± 0.04

[a]Site location is given as latitude (Lat) and longitude (Long). Date of collection (day/month/year) and n are given in the first column. Standard errors (se) are given for $n > 1$. Seuss effect values are calculated from *McCarroll and Loader* [2004] and *Hua et al.* [2013] using a baseline year of 1850.

data set to calculate the fraction of C_4 photosynthesis at a resolution of $3 \times 3°$ ($f_{C4\text{-}9°}$). Our stepwise regression models of Δ included these nine factors: $f_{C4\text{-}1°}$, $f_{C4\text{-}9°}$, CMT-30, WMT-30, MAT, MAT-30, MAP, MAP-30, and pCO_2. Model retention was determined by comparing values of the Akaike information criteria that were corrected for sample size (AICc). The model was then rerun as a linear regression with location as a random effect.

We searched for comparable data in the literature to estimate the effects of climate or pCO_2 on Δ. We used the equation $\Delta = (\delta^{13}C_{CO2} - \delta^{13}C_{substrate})/(1 + \delta^{13}C_{substrate})$. If studies presented data on the relative proportion of C_3 or C_4 vegetation rather than Δ, we assumed that $\Delta_{C3} - \Delta_{C4} = 14.4‰$.

Table 2. AICc and Correlations (r^2) of Different Stepwise Regression Models of Δ[a]

Model	n	r^2	AICc
MAT	1	0.739	221.3
MAT-30	1	0.722	224.3
MAT and MAP-30	2	0.875	188.3
MAT and WMT-30	2	0.864	192.3
pCO_2, MAT, and MAP-30	3	0.915	**172.2**
pCO_2, MAP-30, and WMT-30	3	0.907	176.6
pCO_2, MAT, MAP-30, and WMT-30	4	0.917	**173.7**
pCO_2, MAT, MAP-30, and $f_{C4\text{-}1°}$	4	0.917	**174.0**
pCO_2, MAT, MAP-30, WMT-30, and $f_{C4\text{-}9°}$	5	0.919	175.5
pCO_2, MAT, MAP-30, WMT-30, and MAT-30	5	0.918	176.1

[a]The two models of lowest AICc are shown for each level. Variables included in models included pCO_2, MAT, MAP, MAT-30, MAP-30, WMT-30, CMT-30, $f_{C4\text{-}1°}$, and $f_{C4\text{-}9°}$. $n = 48$. AICc values within 2 of the lowest value are in bold.

Table 3. The Three-Term Regression Model of Δ Values in *Amanita thiersii* That Maximized AICc[a]

Term	%Variance	Estimate ± se	p
Intercept	--	−1.39 ± 10.33	0.8939
MAT (°C)	79.3	−1.74 ± 0.15	<**0.0001**
MAP-30 (m)	15.4	9.52 ± 2.28	**0.001**
pCO_2	5.2	0.072 ± 0.025	**0.0071**

[a]Adjusted r^2 is 0.970, $n = 48$, $p < 0.0001$, with the adjusted r^2 of the fixed effects at 0.910. Location contributed 68% to random effects. "%Variance" is the percentage of variance attributed to the given factor for the regression model run without random effects.

3. Results

In Figure 2, we have plotted sample locations and indicated the annual temperature and precipitation for 2002 to illustrate the broad gradients in temperature and precipitation across the region where *Amanita thiersii* was found. Data on $\delta^{13}C$, pCO_2, the Suess effect, MAT, and MAP are presented in Table 1 by location and year. Latitude and longitude of sampling locations are also given. Sample data (%N, %C, C:N ratio, $\delta^{15}N$, and $\delta^{13}C$) and the percentage of site productivity attributed to C_4 plants are given in supporting information Table S1. Overall, the 48 samples averaged 10.17 ± 1.20 for %N, 40.24 ± 1.97 for %C, 4.01 ± 0.53 for C:N ratio, 10.07 ± 1.59 for $\delta^{15}N$, and −17.65 ± 4.48‰ for $\delta^{13}C$ (±standard deviation).

Results from the stepwise multiple regression on Δ values are given in Table 2. In the stepwise multiple regression, lowest values of AICc were with a three-parameter model that included MAT, MAP-30, and pCO_2 (AICc = 172.2) and a four-factor model that included MAT, MAP-30, pCO_2, and WMT-30 (AICc = 173.7). However, WMT-30 and MAT were highly correlated ($r^2 = 0.988$). The three-factor model explained 91% of the variance in $\delta^{13}C$. Residuals from this regression were correlated within a site, so location was added as a random factor. This increased the explained variance to 97% (Table 3). Sporocarp Δ correlated negatively with MAT (−1.93‰ °C^{-1}, 77% of variance), positively with MAP (0.10‰ cm^{-1}, 15% of variance), and positively with pCO_2 (5% of variance, 0.072‰ ppm^{-1}). The coefficient of 0.072‰ ppm^{-1} for pCO_2 was much higher than the theoretical value of 0.014‰ ppm^{-1} calculated from *Schubert and Jahren* [2015] for C_3 plants. Leverage plots of our three main factors against ^{13}C discrimination are given in Figure 3.

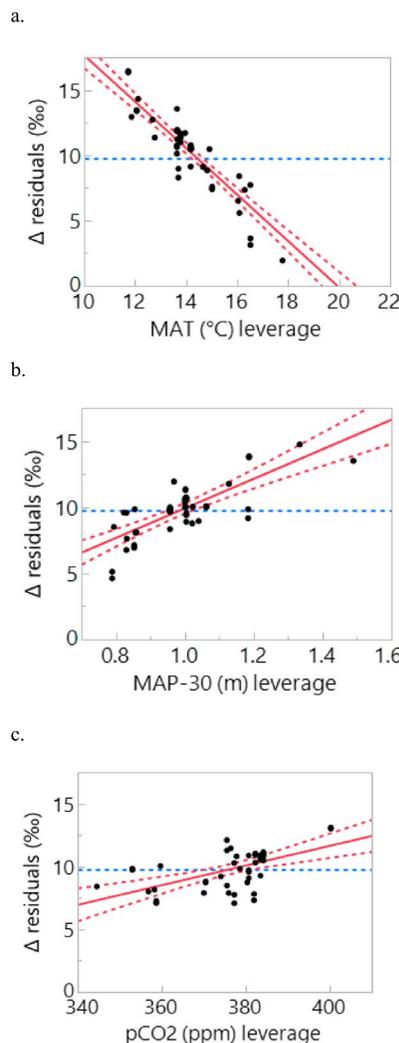**Figure 3.** Leverage plots of the three independent variables versus ^{13}C discrimination (Δ) residuals. (a) Mean annual temperature. (b) Mean annual precipitation. (c) pCO_2.

In Table 4, we compared our coefficients of the effects of pCO_2, temperature, and precipitation on Δ from estimates

Table 4. Estimated Effects of pCO$_2$, MAT, and MAP on Δ Calculated From Prior Studies[a]

pCO$_2$ (‰ ppm^{-1})	Temperature (‰ °C^{-1})	Precipitation (‰ m^{-1})	Study	Notes
0.0064	−0.10[b]	0.31[c]	Kö	C$_3$ grassland, summer/fall, 1875–2007
0.0015 (ns)	−0.11[d]	1.17[e]	Kö	C$_3$ grassland, spring, 1857–2007
0.014	--	--	SJ	elevated CO$_2$ studies, C$_3$ plants
0.072	−1.74	9.52	Ho	current study
--	−0.75[f]	--	PL	USA climate gradients, C$_3$/C$_4$ vegetation
--	−0.49[g], 0.78[h]	--	vF	USA climate gradients, C$_3$/C$_4$ A horizon roots
--	−0.65[i]	--	Au	Mongolian sheep wool from C$_3$/C$_4$ pastures

[a]In some studies, parameters were referenced relative to the proportional shift in C$_3$ versus C$_4$ plant productivity or soil. We have converted those data to reflect shifts in Δ by assuming that C$_3$ plants average 14.4‰ higher in Δ than C$_4$ plants. Calculations are given in supporting information Table S4. Cited studies: *Köhler et al.* [2010] (Kö), *Schubert and Jahren* [2012] (SJ), current study (Ho), *Paruelo and Lauenroth* [1996] (PL), *von Fischer et al.* [2008] (vF), and *Auerswald et al.* [2012] (Au). ns, not significant.
[b]August mean temperature.
[c]Log of September rainfall in mm d^{-1}, here converted to m yr^{-1} using 0.61 m yr^{-1} and 0.855 m yr^{-1} as representative values (MAP was 0.735 ± 0.120 m).
[d]June mean temperature.
[e]Log of March–June rainfall in mm d^{-1}, here converted to m yr^{-1} using 0.61 m yr^{-1} and 0.855 m yr^{-1} as representative values (MAP was 0.735 ± 0.120 m).
[f]Estimated from multiple regression, MAT, as given in supporting information Tables S2, S3, and S4.
[g]MAT.
[h]April through August temperature. [i]July temperature.

derived from previously published work in elevated CO$_2$ experiments, archived long-term studies, and studies of controls over either C$_3$ and C$_4$ plants or C$_3$- and C$_4$-derived soils. Our calculations are presented in supporting information Table S4. Coefficient estimates were higher in our study than in other work (Table 4).

4. Discussion

Carbon from *Amanita thiersii* reflected an overall balance between C$_3$ and C$_4$ photosynthesis over long periods at a site (Table 3). In our regression, the strong correlation of Δ with mean annual temperature presumably reflects an underlying correlation between MAT and the dominance of C$_4$ grasses [*Tieszen et al.*, 1997], but we assume that this pattern in turn reflects human selection for turf grass species that are C$_3$ or C$_4$ [*Milesi et al.*, 2005] and subsequent competition among planted and local species, rather than competition within natural vegetation. High temperatures during the growing season rather than mean annual temperatures appear to control C$_3$ versus C$_4$ grass distributions in studies of natural grasslands; for example, the best single predictor of %C$_4$ in soil organic matter in the A horizon was the high monthly temperature for April through August [*von Fischer et al.*, 2008]. However, mean annual temperature will correlate closely in most cases with the high monthly temperature [*Ehleringer et al.*, 1997] and in the current study the correlation between mean annual temperature and the mean temperature of the warmest summer month (WMT-30) correlated very closely, with $r = 0.994$. Growing season temperatures will also largely control the choice of C$_3$ and C$_4$ turf species for a specific location [*Bertrand et al.*, 2013], in addition to other factors such as freezing tolerance [*Dionne et al.*, 2010].

As in studies focusing on natural vegetation [*von Fischer et al.*, 2008], MAP was a significant factor influencing Δ. Lawn turf grasses are selected for their drought tolerance [*Bonos and Huff*, 2013], with C$_4$ grasses more drought tolerant than C$_3$ grasses. Thus, regional shifts in MAP should increase the proportion in lawns of C$_4$ grasses of low Δ. Prior conditions, as potentially indicated by f_{C4}, did not significantly influence Δ, perhaps implying that *Amanita thiersii* carbon is drawn primarily from recent photosynthate. However, the C$_4$ distribution in *Still et al.* [2009] was driven by climate plus the economic incentives to grow maize, an annual C$_4$ crop, in regions where the C$_4$ perennial grasses used in lawns would overwinter poorly. Thus, it is not surprising that temperature and precipitation were better predictors of Δ in our study than large-scale estimates of C$_4$ distribution that included maize cultivation.

We point out that in the regression model, MAT and MAP-30 were selected, rather than MAT-30 and MAP. MAP correlated strongly with MAP-30 ($r = 0.898$), but we assume that MAP-30 is a better representation of

the long-term average of precipitation that influences C_3 and C_4 grass productivity than an individual year, even though MAP-30 is the average from 1961 to 1990, whereas samples were from 1982 to 2009. In contrast, calculated MAT in our study correlated so strongly with MAT-30 ($r = 0.994$) that they were essentially identical in their effects on the regression model, and, in fact, they were the two most important single factors in the one-factor regression model (Table 2).

Comparable values for our coefficients of our regression model from the literature are given in Table 4. Not surprisingly, in the two studies of exclusively C_3 plants [*Köhler et al.*, 2010; *Schubert and Jahren*, 2012], the estimated coefficients for pCO_2, temperature, and precipitation are ~10 times lower than in our study that looked across C_3/C_4 gradients. It is unclear why the temperature coefficient is higher in our study than in the three other studies assessing this across natural C_3/C_4 gradients. We suspect that summertime lawn watering, by favoring photosynthesis of C_4 grasses under hot summertime conditions, could extend the relative dominance of C_4 grasses northward until they are limited by cold winter temperatures, thereby effectively sharpening the temperature gradient of the C_3/C_4 transition. Turf management in the U.S. divides the country into different regions, with C_3, cool-season grasses predominating northward and C_4, warm-season grasses predominating in the south [*Christians and Engelke*, 1994]. The relatively narrow transition zone between these two regions may be where a mix of C_3 and C_4 grasses can survive and may account for the steep temperature gradient recorded in our study. Our estimate of $1.93‰$ $°C^{-1}$ translates into about a $7.5°C$ increase in MAT from 100% C_3 to 100% C_4, if we assume that $\Delta_{C3\text{-}C4}$ is $14.4‰$.

The following equation modified from *Schubert and Jahren* [2015] predicted that the pCO_2 increase across the study interval (1982 to 2009) should increase Δ by $0.64‰$ in C_3 plants by decreasing photorespiration:

$$pCO_2 \text{ effect} = \left[A \times B \times \left(pCO_{2(2009)} + C\right)\right] / \left[A + B \times \left(pCO_{2(2009)} + C\right)\right]$$
$$- \left[A \times B \times \left(pCO_{2(1982)} + C\right)\right] / \left[A + B \times \left(pCO_{2(1982)} + C\right)\right] \tag{1}$$

where $A = 28.26$, $B = 0.22$, and $C = 23.9$, $pCO_{2(1982)} = 341$ ppm, and $pCO_{2(2009)} = 387$ ppm. Given a 47 ppm shift over this period ($pCO_2 = 341$ ppm in 1982 and 387 ppm in 2009), the theoretical coefficient for the photorespiratory effect in C_3 plants would be $0.64‰/46$ ppm or $0.014‰/ppm$. However, the observed coefficient estimate is $0.072‰/ppm$ (Table 2), meaning that pCO_2 alone cannot account for the increased Δ; additional factors are therefore required to explain the increase in Δ. One likely possibility for the increased Δ across the study interval is increased assimilation by *Amanita thiersii* of C_3-derived carbon, presumably resulting from increased productivity of C_3 grasses relative to C_4 grasses and lower photorespiratory losses in C_3 grasses with rising CO_2 concentrations [*Wand et al.*, 1999]. Thus, the observed Δ increase in *Amanita thiersii* probably reflected both decreased photorespiration with rising pCO_2 and shifts in the competitive balance between C_3 and C_4 grasses in suburban lawns.

We suggest that the competitive balance has shifted toward C_3 grasses under these conditions sufficiently to increase the coefficient for pCO_2 in our regression model by a factor of 5 (from the calculated value for the photorespiratory effect of $0.014‰$ ppm^{-1} to $0.072‰$ ppm^{-1}). From 1982 to 2009, this corresponded to a $2.67‰$ increase in Δ of *Amanita*-assimilated carbon driven by the productivity shift to C_3 photosynthesis, as calculated from $(0.072‰–0.014‰)$ $ppm^{-1} \times 46$ ppm. With an assumed $14.4‰$ difference in Δ between C_3 and C_4 photosynthesis [*O'Leary*, 1988], the calculated shift corresponded to a 18.5% (2.67/14.4) increase in the proportion of total productivity attributed to C_3 grasses over the 46 ppm increase in pCO_2 This corresponded to a 40% increase per 100 ppm pCO_2 increase.

We can compare these field-derived results against those estimated from numerous experiments on C_3 plants, which showed that biomass increased on average by 20–54% as pCO_2 increased from ambient (300–420 ppm) to elevated (475–700 ppm) levels [*Ainsworth and Long*, 2005; *Kimball et al.*, 1993; *Poorter*, 1993; *Poorter and Navas*, 2003; *Wand et al.*, 1999]. Biomass of C_3 grasses (Poaceae) specifically increased by 33% to 44% [*Wand et al.*, 1999]. Using these results as a guide, a 40% increase in biomass in response to a 130 ppm increase in pCO_2 (e.g., 370 to 500 ppm) suggests a 31% increase in biomass per 100 ppm increase in pCO_2, which is similar to that determined here for lawns (40% per 100 ppm).

We note, however, that the pCO_2 levels for our study spanned a narrow range and were all less than 400 ppm (341 to 387 ppm). Given that the biomass response is greatest at low pCO_2 and saturates at higher pCO_2 [*Hunt*

et al., 1991, 1993; *Schubert and Jahren*, 2011], it is not surprising that the response we observed at low pCO$_2$ is greater than that observed within studies that included significantly higher pCO$_2$ levels (e.g., up to 750 ppm in the *Wand et al.* [1999] review). Thus, the integrated growth response that we have estimated in this study using *Amanita thiersii* as an integrator of lawn photosynthesis appears entirely plausible based on numerous growth studies of C$_3$ grasses under elevated pCO$_2$. Although shifts in lawn maintenance practices could also influence Δ, the physiological responses of turf grasses since the early 1980s to rising pCO$_2$ could readily account for the integrated patterns reported here.

5. Conclusions

Amanita thiersii appeared to be a good integrator of the carbon produced by grasses in lawns. Temperature was the primary control over C$_3$ versus C$_4$ grass distribution. Both management decisions and competition between grass types within lawns could play a role in the patterns of carbon assimilated by *Amanita thiersii*. Rising pCO$_2$ over the 1982–2009 record correlated with the relative C$_3$ and C$_4$ assimilation by this fungus, suggesting that rising pCO$_2$ may have significantly affected the competitive balance between the two grass types. This suggests that herbarium specimens of sporocarps could be used more widely to examine ecosystem-scale responses to global change.

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