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RESEARCH ARTICLE

10.1002/2016JG003579

Key Points:

- Carbon isotopes in a lawn fungus integrated C₃ and C₄ productivity in lawns between 1982 and 2009
- Higher temperatures favored C₄ productivity, and higher precipitation favored C₃ productivity
- Between 1982 and 2009, pCO₂ increased by 47 ppm and the relative productivity of C₃ grasses increased 18.5%

Supporting Information:

- Supporting Information S1

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Increased C₃ productivity in Midwestern lawns since 1982 revealed by carbon isotopes in *Amanita thiersii*

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Abstract How climate and rising carbon dioxide concentrations (pCO₂) have influenced competition between C₃ and C₄ plants over the last 50 years is a critical uncertainty in climate change research. Here we used carbon isotope (δ¹³C) values of the saprotrophic lawn fungus *Amanita thiersii* to integrate the signal of C₃ and C₄ carbon in samples collected between 1982 and 2009 from the Midwestern USA. We then calculated ¹³C fractionation (Δ) to assess the balance between C₃ and C₄ photosynthesis as influenced by mean annual temperature (MAT), mean annual precipitation over a 30 year period (MAP-30), and pCO₂. 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 C₃ and C₄ grasses in lawns. In addition, Δ values correlated positively with pCO₂ (0.072‰ ppm^{−1}, 5% of variance). Reduced photorespiration with rising pCO₂ accounted for 20% of this increased Δ, but the remaining 80% is consistent with increased assimilation of C₃-derived carbon by *Amanita thiersii* resulting from increased productivity of C₃ grasses with rising pCO₂. Between 1982 and 2009, pCO₂ rose by 46 ppm and the relative contribution of C₃ photosynthesis to *Amanita thiersii* carbon increased 18.5%. The δ¹³C value of *Amanita thiersii* may integrate both lawn maintenance practices and the physiological responses of turf grasses to rising CO₂ concentrations.

1. Introduction

Fundamental differences between the C₃ and C₄ modes of photosynthesis account for the higher ¹³C:¹²C ratios (expressed as δ¹³C values) observed in C₄ plants than in C₃ plants. These fundamental differences also explain the differential responses of C₄ and C₃ grasslands to changes in atmospheric carbon dioxide levels (pCO₂), temperature, precipitation, and nutrient availability. In mixed communities, the competitive balance between C₃ and C₄ grasses should accordingly be sensitive to climate change and ongoing increases in pCO₂. Researchers have used δ¹³C values in grazers to examine the balance between C₃ and C₄ grasses in paleontological studies stretching back 5–8 Ma [Cerling *et al.*, 1997] and have done similar studies in modern grasslands [Auerwald *et al.*, 2012], although data are needed on responses to the sharply rising pCO₂ 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 C₃ versus C₄ 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 km² in 2005 (Figure 1) [Steinberg, 2006]. The δ¹³C values of *Amanita thiersii* indicated that it can assimilate carbon derived from both C₃ and C₄ photosynthetic pathways [Wolfe *et al.*, 2012]. Here we will use isotopic patterns in *Amanita thiersii* to integrate local productivity of C₃ versus C₄ grasses and relate that productivity to climatic factors and pCO₂.

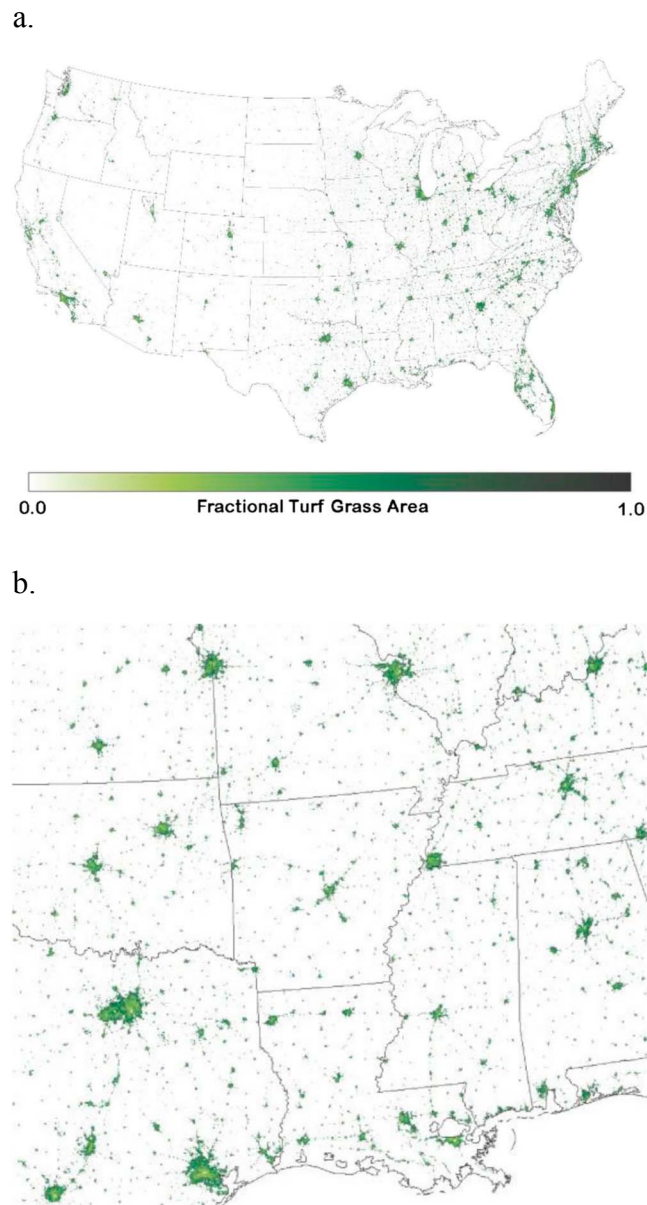


Figure 1. Fraction of USA surface area covered by lawns. From http://eoi-images.gsfc.nasa.gov/images/imagerecords/6000/6019/frac_turf_grass_lrg.jpg. (a) United States. (b) Sampling area in south central USA.

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 $\delta^{13}\text{C}$ 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 $p\text{CO}_2$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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, $p\text{CO}_2$, and the regionally estimated proportion of C_4 photosynthesis (f_{C_4}) to

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_{C_4}) 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 $\delta^{13}\text{C}$ of atmospheric CO_2 caused by the burning of fossil fuels since the start of the Industrial Revolution, also influences $\delta^{13}\text{C}$ patterns. The $\delta^{13}\text{C}$ of atmospheric CO_2 has decreased by about 1.7‰ since 1850, while $p\text{CO}_2$ has risen from

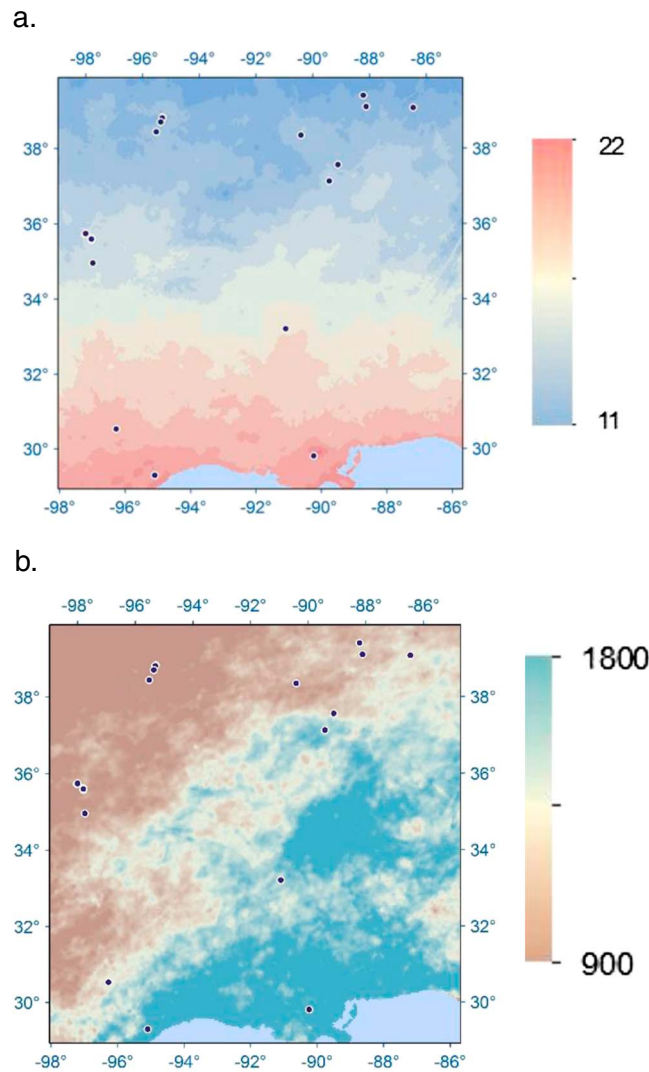


Figure 2. Collection locations are plotted by latitude (°N) and longitude (°W, given a negative sign) versus (a) mean annual temperature, MAT, and (b) mean annual precipitation, MAP. Colored isopleths are 1°C for temperature (from 11°C to 22°C) and 200 mm for precipitation (from 900 mm to 1800 mm). Isopleths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

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 $\delta^{13}\text{C}_{\text{CO}_2}$ and $p\text{CO}_2$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\Delta = (\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{Amanita}}) / (1 + \delta^{13}\text{C}_{\text{Amanita}})$. 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 ($f_{\text{C}_4-1^\circ}$). We also used the *Still et al.* [2009]

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. $\delta^{13}\text{C}$ 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_{C_4} , temperature, and precipitation.
2. Because $p\text{CO}_2$ 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, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ 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

Table 1. Data on Annual $p\text{CO}_2$, the Suess Effect, Mean Annual Temperature (MAT), Mean Annual Precipitation (MAP), and $\delta^{13}\text{C}^a$

| Date (n) | $p\text{CO}_2$ (ppm) | Suess Effect (‰) | Lat (°N) | Long (°W) | MAT (°C) | MAT-30 (°C) | MAP (mm) | MAP-30 (mm) | $\delta^{13}\text{C} \pm \text{se}$ (‰) |
|---------------|----------------------|------------------|----------|-----------|----------|-------------|----------|-------------|---|
| 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 |

^aSite 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 $n > 1$. Suess 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 $3 \times 3^\circ$ ($f_{C_4-9^\circ}$). Our stepwise regression models of Δ included these nine factors: $f_{C_4-1^\circ}$, $f_{C_4-9^\circ}$, CMT-30, WMT-30, MAT, MAT-30, MAP, MAP-30, and $p\text{CO}_2$. 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 $p\text{CO}_2$ on Δ . We used the equation $\Delta = (\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C}_{\text{substrate}})/(1 + \delta^{13}\text{C}_{\text{substrate}})$. If studies presented data on the relative proportion of C_3 or C_4 vegetation rather than Δ , we assumed that $\Delta_{C_3} - \Delta_{C_4} = 14.4\text{‰}$.

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 |
| $p\text{CO}_2$, MAT, and MAP-30 | 3 | 0.915 | 172.2 |
| $p\text{CO}_2$, MAP-30, and WMT-30 | 3 | 0.907 | 176.6 |
| $p\text{CO}_2$, MAT, MAP-30, and WMT-30 | 4 | 0.917 | 173.7 |
| $p\text{CO}_2$, MAT, MAP-30, and $f_{C_4-1^\circ}$ | 4 | 0.917 | 174.0 |
| $p\text{CO}_2$, MAT, MAP-30, WMT-30, and $f_{C_4-9^\circ}$ | 5 | 0.919 | 175.5 |
| $p\text{CO}_2$, MAT, MAP-30, WMT-30, and MAT-30 | 5 | 0.918 | 176.1 |

^aThe two models of lowest AICc are shown for each level. Variables included in models included $p\text{CO}_2$, MAT, MAP, MAT-30, MAP-30, WMT-30, CMT-30, $f_{C_4-1^\circ}$, and $f_{C_4-9^\circ}$. $n = 48$. 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 \pm se | <i>p</i> |
|--------------------------|-----------|-------------------|----------|
| 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 |
| <i>p</i> CO ₂ | 5.2 | 0.072 ± 0.025 | 0.0071 |

^aAdjusted 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 $\delta^{13}\text{C}$, *p*CO₂, 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, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$) and the percentage of site productivity attributed to C₄ 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 $\delta^{15}\text{N}$, and $-17.65 \pm 4.48\text{‰}$ for $\delta^{13}\text{C}$ (\pm 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 *p*CO₂ (AICc = 172.2) and a four-factor model that included MAT, MAP-30, *p*CO₂, 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 $\delta^{13}\text{C}$. 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 ($-1.93\text{‰}\text{°C}^{-1}$, 77% of variance), positively with MAP ($0.10\text{‰}\text{cm}^{-1}$, 15% of variance), and positively with *p*CO₂ ($0.072\text{‰}\text{ppm}^{-1}$, 5% of variance, $0.072\text{‰}\text{ppm}^{-1}$). The coefficient of $0.072\text{‰}\text{ppm}^{-1}$ for *p*CO₂ was much higher than the theoretical value of $0.014\text{‰}\text{ppm}^{-1}$ calculated from Schubert and Jahren [2015] for C₃ plants. Leverage plots of our three main factors against $\delta^{13}\text{C}$ discrimination are given in Figure 3.

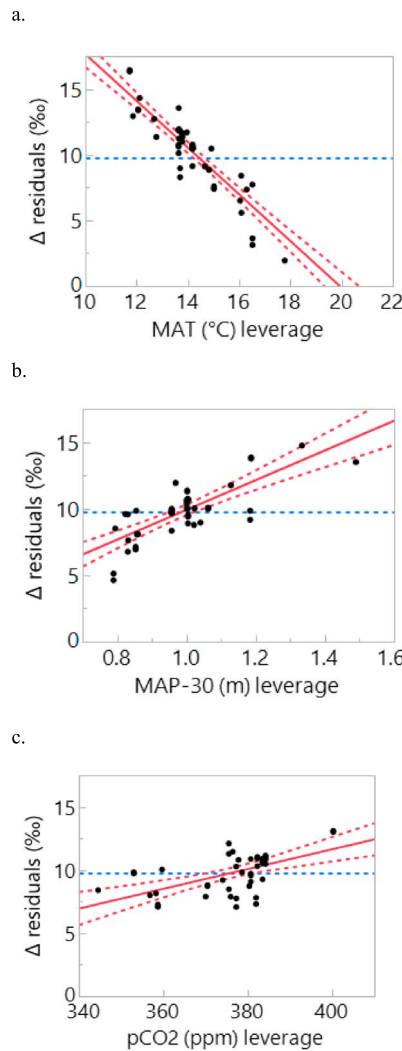


Figure 3. Leverage plots of the three independent variables versus $\delta^{13}\text{C}$ discrimination (Δ) residuals. (a) Mean annual temperature. (b) Mean annual precipitation. (c) *p*CO₂.

In Table 4, we compared our coefficients of the effects of *p*CO₂, temperature, and precipitation on Δ from estimates

Table 4. Estimated Effects of $p\text{CO}_2$, MAT, and MAP on Δ Calculated From Prior Studies^a

| $p\text{CO}_2$ (%o ppm ⁻¹) | Temperature (%o °C ⁻¹) | Precipitation (%o 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 |

^aIn some studies, parameters were referenced relative to the proportional shift in C₃ versus C₄ plant productivity or soil. We have converted those data to reflect shifts in Δ by assuming that C₃ plants average 14.4%o higher in Δ than C₄ 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.

^bAugust mean temperature.

^cLog 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).

^dJune mean temperature.

^eLog 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).

^fEstimated from multiple regression, MAT, as given in supporting information Tables S2, S3, and S4.

^gMAT.

^hApril through August temperature. ⁱJuly temperature.

derived from previously published work in elevated CO₂ experiments, archived long-term studies, and studies of controls over either C₃ and C₄ plants or C₃- and C₄-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 C₃ and C₄ 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 C₄ grasses [Tieszen *et al.*, 1997], but we assume that this pattern in turn reflects human selection for turf grass species that are C₃ or C₄ [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 C₃ versus C₄ grass distributions in studies of natural grasslands; for example, the best single predictor of %C₄ 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 C₃ and C₄ 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 C₄ grasses more drought tolerant than C₃ grasses. Thus, regional shifts in MAP should increase the proportion in lawns of C₄ grasses of low Δ . Prior conditions, as potentially indicated by f_{C_4} , did not significantly influence Δ , perhaps implying that *Amanita thiersii* carbon is drawn primarily from recent photosynthate. However, the C₄ distribution in Still *et al.* [2009] was driven by climate plus the economic incentives to grow maize, an annual C₄ crop, in regions where the C₄ 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 C₄ 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 $1.93\text{‰} \text{ }^\circ\text{C}^{-1}$ translates into about a 7.5°C increase in MAT from 100% C_3 to 100% C_4 , if we assume that $\Delta_{C_3-C_4}$ 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:

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

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 $0.64\text{‰}/46$ ppm or $0.014\text{‰}/\text{ppm}$. However, the observed coefficient estimate is $0.072\text{‰}/\text{ppm}$ (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 $0.014\text{‰} \text{ ppm}^{-1}$ to $0.072\text{‰} \text{ ppm}^{-1}$). 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 $(0.072\text{‰} - 0.014\text{‰}) \text{ ppm}^{-1} \times 46$ 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 $20\text{--}54\%$ 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 $p\text{CO}_2$ is greater than that observed within studies that included significantly higher $p\text{CO}_2$ 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 $p\text{CO}_2$. Although shifts in lawn maintenance practices could also influence Δ , the physiological responses of turf grasses since the early 1980s to rising $p\text{CO}_2$ 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 $p\text{CO}_2$ over the 1982–2009 record correlated with the relative C_3 and C_4 assimilation by this fungus, suggesting that rising $p\text{CO}_2$ 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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