Appendix A: Observed Spatial and Temporal Variability of $\delta_{L,b}$

Variations of the isotopic enrichment with leaf position

In this section, we summarize the key features of the spatial and temporal variability of $\delta_{L,b}$ observed in the field experiment. There were large diurnal variations in the gradient of the isotopic enrichment along the leaf (Fig. A1). If the whole leaf was considered, the leaf isotopic enrichment was weak in the early morning, became stronger in midday and decreased in the evening, with a whole-leaf $\delta_{L,b}$ value of -2.7‰, 2.9‰, 12.6‰, 12.1‰ to 8.1‰ at 6:37, 9:21, 12:51, 15:22 and 18:21 LST, respectively. (The δ_x and δ_a values on the observational day were -8.5 and -14.1‰, respectively.) In addition, it was worth noting that the whole-leaf mean $\delta_{L,b}$ was close to that at the middle leaf position.

An obvious diurnal variation of the progressive isotopic enrichment with leaf position was observed. In the morning, there was little isotopic gradient along the leaf. In the afternoon, an isotopic gradient from the leaf base to the tip was established, with a gradient value of 15.3 and 15.2‰ (tip-to-base) at 12:51 and 15:22 LST, respectively. In the early evening, the gradual enrichment was maintained, with the gradient decreased to 11.3‰ (tip-to-base) at 18:21 LST.

Humidity was a major factor controlling the isotopic gradient along the leaf. There was a significant negative correlation between the $\delta_{L,b}$ gradient (tip-to-base) and relative humidity (Fig. A2), with r = -0.97 (number of observations n = 5, p < 0.01). When relative humidity was low (~40%) in the afternoon, the gradient was high (~15.‰). In the early morning, the gradient was slightly negative (~ -0.9‰) when relative humidity was high (~96%).

Variations of the isotopic enrichment in the canopy

Within the canopy stratification, the enrichment extent of the upper leaves was different from that of the lower leaves for both wheat and corn (Figs. A3 and A4). Over the wheat growing season, the midday $\delta_{L,b}$ of the leaves in the upper canopy was always greater than that in the lower canopy. The seasonal variation of the upper-to-lower canopy isotopic gradient had the similar pattern to the LAI variation. Over the period from DOY 113 to 142 when LAI was large (> 3.5), the isotopic gradient was also large, with a mean value of 2.9‰. For comparison, the gradient was smaller at the beginning and the end of the growth season when LAI was smaller. The maximum difference (upper-to-lower canopy) was 6.7‰ on DOY 133, and the minimum was around 0 on DOY 95 and 160. A close correlation was found between the upper-to-lower canopy gradient of $\delta_{L,b}$ and the gradient of leaf temperature, with $r = 0.56$ ($n = 14$, $p < 0.05$). During the intensive periods when leaf physiological measurement was available, the midday leaf temperature in the upper layer was usually higher than in the lower layer, with the difference (upper-to-lower canopy) ranging from 0.3 to 0.8 ^{o}C.

Diurnal variations of the upper-to-lower canopy $\delta_{L,b}$ gradient were observed in wheat during the intensive periods (Fig. A4). In the early morning, the enrichment was similar between the upper and the lower leaves, with the difference ranging from -1.3‰ (DOY 143) to 1.6‰ (DOY 140). At other times, the upper leaves were more enriched than the lower leaves, and the largest gradient occurred in the late morning to the early afternoon.

For corn, the midday $\delta_{L,b}$ of the upper leaves was lower than that of the lower leaves before LAI and canopy height reached their maximum values on DOY 229. Afterwards, the pattern was reversed, with the upper leaves becoming more enriched than the lower leaves (Fig. A3). The seasonal variation of the midday $\delta_{L,b}$ gradient (upper to lower canopy) ranged from -7.6‰ (DOY 188) to 3.9‰ (DOY 255), with a seasonal mean value of -0.4‰. The same pattern reversals were also found in soybean by Welp *et al*. (2008) and in corn by Griffis *et al*. (2011) in the Upper Middest of USA; these authors suggested that the differences in humidity and the kinetic fractionation effect between the upper and lower canopy may be contributing factors. According to the simultaneous physiology measurements, a close relationship was found between the midday upper-to-lower canopy isotopic gradient with the stomatal resistance gradient (r = -0.78, n = 15, p <0.001). The stomatal resistance of the upper leaves was lower than that of the lower leaves, with the difference ranging from -0.3 to -13.1 m^2 s mol^{-1} (upper-to-lower canopy) and a mean difference of -5.3 m^2 s mol^{-1}. It is worth mentioning that the resistance gradient was small before LAI reached its maximum, with the mean value of -2.5 m^2 s mol^{-1} before the maximum, in comparison to the mean gradient of -9.5 m^2 s mol^{-1} afterwards.

In terms of the diurnal pattern of the $\delta_{L,b}$ gradient (upper-to-lower canopy) in corn canopy, the upper leaves were usually more enriched than lower leaves in the daytime and less enriched at night. For example, the $\delta_{L,b}$ gradient was positive before 18:30 on DOY 246 and negative at other times.

Appendix B. Correction of IRIS Measurements for organic contamination

The spectral contaminations of ethanol and methanol on the IRIS measurements were corrected according to the method proposed by Schultz *et al*. (2011). Briefly, the broadband (BB, for EtOH) and narrowband (NB, for MeOH) contamination metrics were established for our IRIS analyzer (Fig. A5). The relationship between the offset in δ^{18}O and the metrics were then used to correct the contaminations of EtOH and MeOH. The parameters of these correction relationships were quite different from those obtained by Schultz *et al*. (2011), indicating that no universal correction curves exist. Schultz *et al*. (2011) showed that precipitation and soil water samples are not prone to the organic contamination. In this study, the isotope values of the stem and leaf samples were corrected using the correction curves (Table A1).

For independent verification of the correction method, a subset of the leaf samples were measured with IRMS (16 wheat stem, 14 corn stem, 63 wheat leaf and 70 corn leaf samples; Fig. A6). The bias error (IRIS minus IRMS) was 2.5‰ ± 2.0‰ (mean ± one standard deviation) prior to and -0.3‰ ± 0.7‰ after the correction.

Table A1 Contamination correction to the $\delta^{18}O$ values of wheat and corn leaves and stems.

Sample name	Mean $\delta^{18}O$(‰)		
	Before correction	$\Delta\delta^{18}O$	After correction
wheat stems	-5.6	1.0 ± 0.6	-6.6
corn stems	-6.1	1.4 ± 1.0	-7.5
wheat leaves	6.1	2.2 ± 1.1	3.9
corn leaves	6.0	2.8 ± 1.2	3.2

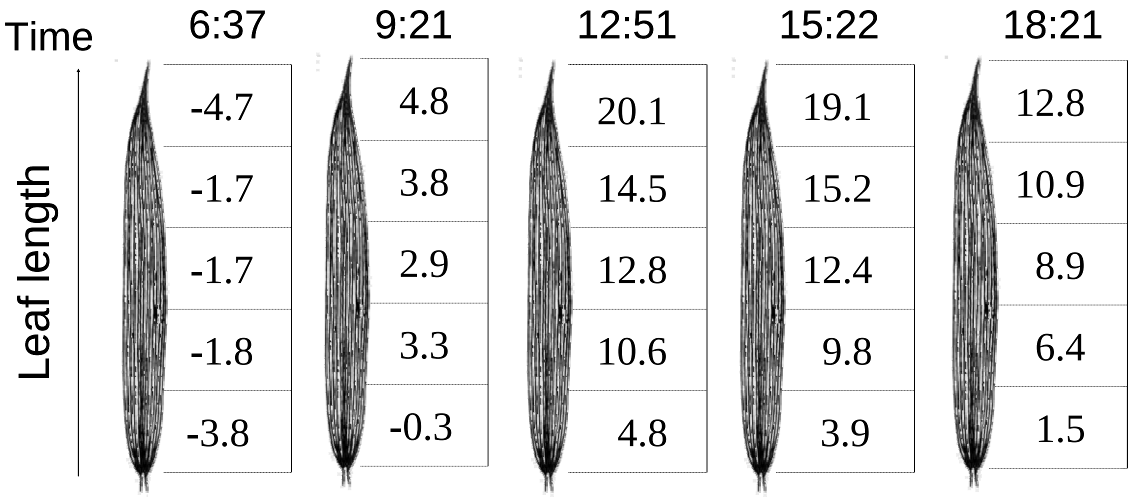
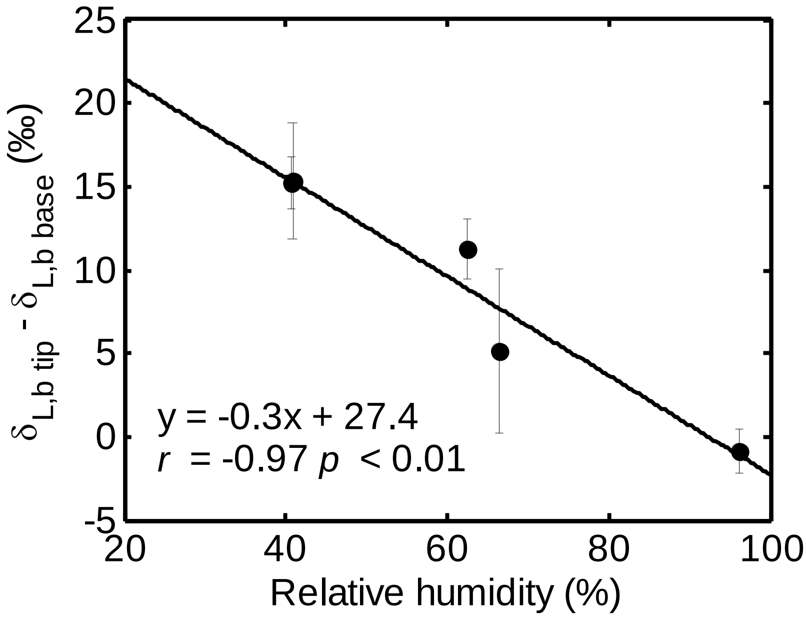

Time
6:37
9:21
12:51
15:22
18:21
Leaf length
-4.7
-1.7
-1.7
-1.8
-3.8
4.8
3.8
2.9
3.3
-0.3
20.1
14.5
12.8
10.6
4.8
19.1
15.2
12.4
9.8
3.9
12.8
10.9
8.9
6.4
1.5

Fig. A1



25
20
15
10
5
0
-5
20
40
60
80
100
Relative humidity (%)
δL,b tip - δL,b base (‰)
y = -0.3x + 27.4
r = -0.97 p < 0.01

Fig. A2

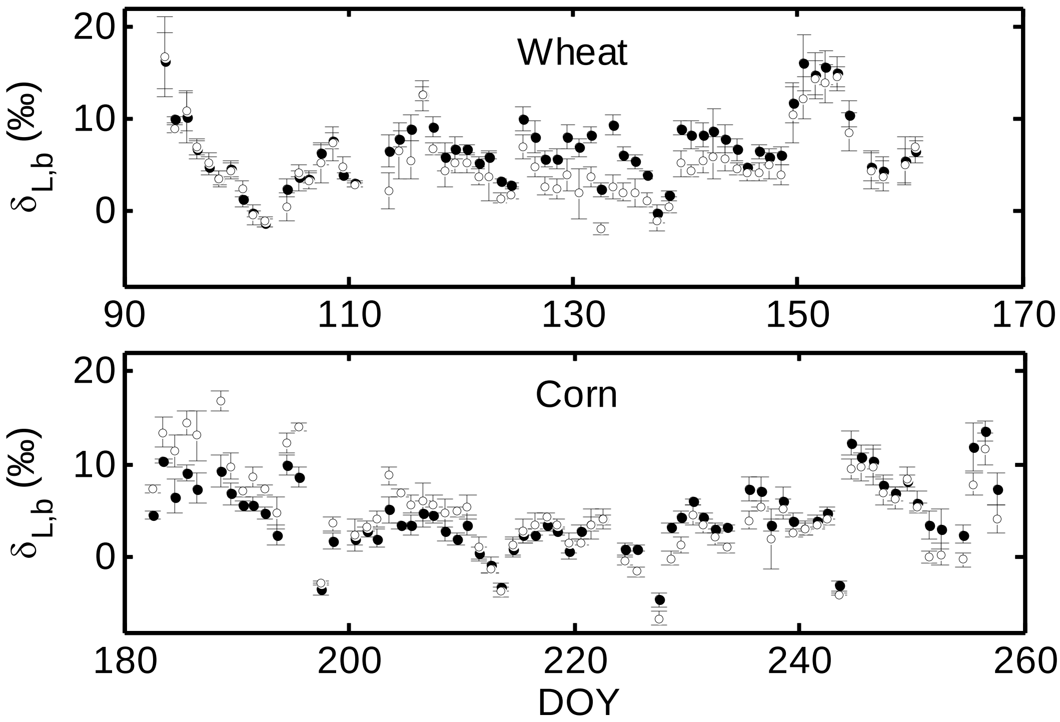

Wheat
Corn
$\delta_{L,b}$ (‰)
20
10
0
90
110
130
150
170
180
200
220
240
260
DOY

Fig. A3

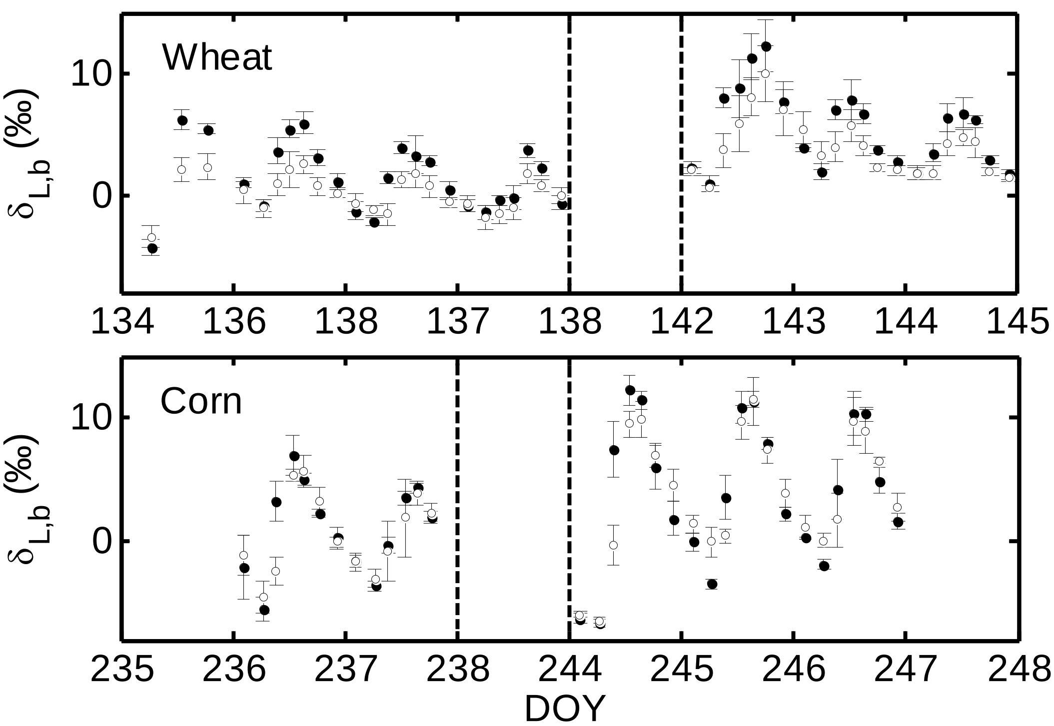

Wheat
Corn
δL,b (‰)
10
0
134
136
138
137
138
142
143
144
145
235
236
237
238
244
245
246
247
248
DOY

Fig. A4

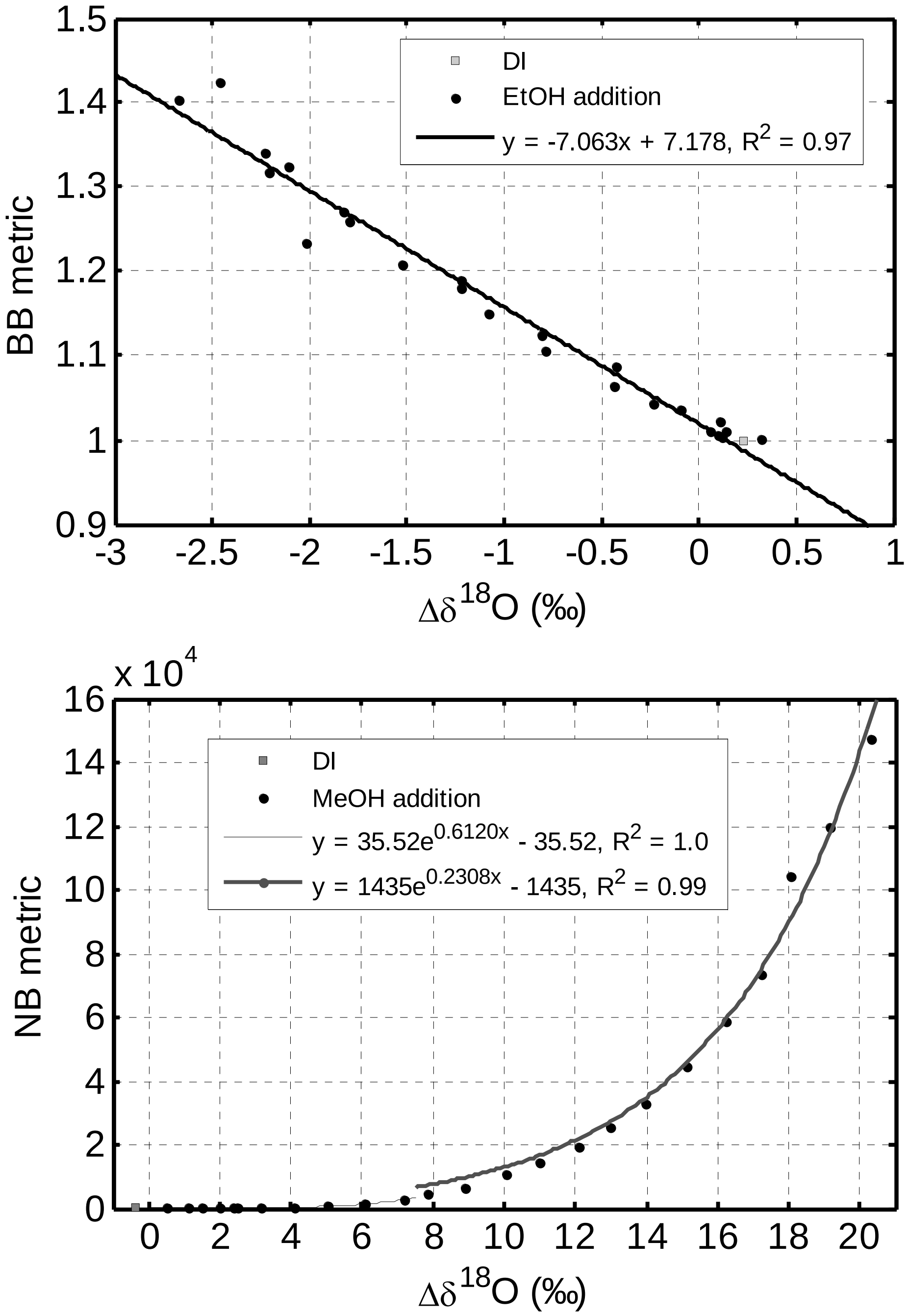

1.5
1.4
1.3
1.2
1.1
1
0.9
BB metric
DI
EtOH addition
y = -7.063x + 7.178, R2 = 0.97
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
Δδ18O (‰)
x 104
16
14
12
10
8
6
4
2
0
NB metric
DI
MeOH addition
y = 35.52e0.6120x - 35.52, R2 = 1.0
y = 1435e0.2308x - 1435, R2 = 0.99
0
2
4
6
8
10
12
14
16
18
20
Δδ18O (‰)

Fig. A5

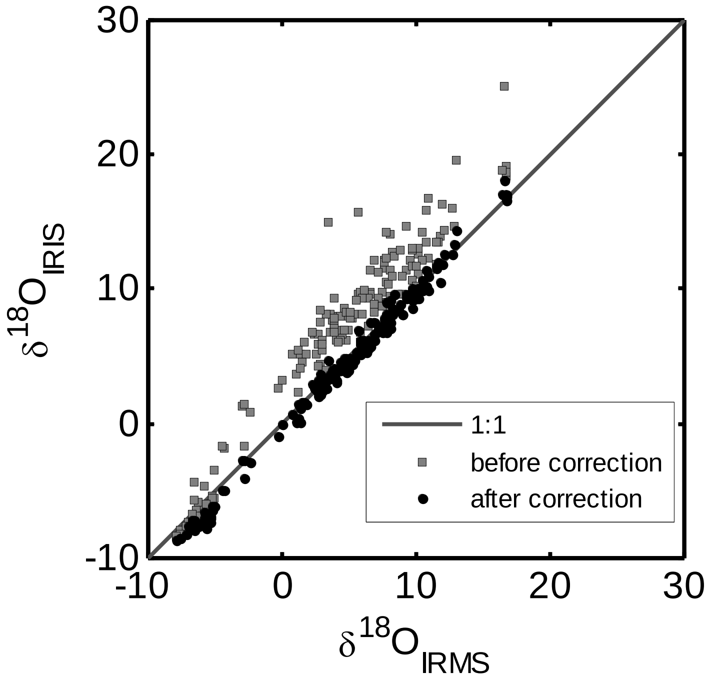

30
20
10
0
-10
-10
0
10
20
30
δ18OIRIS
δ18OIRMS
1:1
before correction
after correction

Fig. A6