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Supplement of

Determination of respiration and photosynthesis fractionation factors for atmospheric dioxygen inferred from a vegetation–soil–atmosphere analogue of the terrestrial biosphere in closed chambers

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Table S1. Succession of dark and light periods for the different sequences of the photosynthesis and dark respiration experiment.

Sequence	Light	Start date	End date
1	On	19/03/19, 08:00	25/03/19, 14:00
	Off	25/03/19, 14:00	28/03/19, 17:05
	On	28/03/19, 17:05	02/04/19, 08:00
	Off	02/04/19, 08:00	05/04/19, 06:50
	On	05/04/19, 06:50	16/04/19, 15:30
	Off	16/04/19, 15:30	19/04/19, 06:50
	On	19/04/19, 06:50	06/05/19, 14:00
	Off	06/05/19, 14:00	14/05/19, 14:20
	On	14/05/19, 14:20	15/05/19, 14:00
2	On	20/05/19,0 6:00	28/05/19, 13:00
	Off	28/05/19, 13:00	30/05/19, 20:35
	On	30/05/19, 20:35	10/06/19, 11:00
	Off	10/06/19, 11:00	14/06/19, 15:25
	On	14/06/19, 15:25	23/06/19, 14:30
	Off	23/06/19, 14:30	27/06/19, 05:25
	On	27/06/19, 05:25	28/06/19, 08:35
3	On	29/07/19, 07:00	05/08/19, 14:00
	Off	05/08/19, 14:00	08/08/19, 05:20
	On	08/08/19, 05:20	19/08/19, 13:00
	Off	19/08/19, 13:00	22/08/19, 05:25
	On	22/08/19, 05:25	02/09/19, 13:00
	Off	02/09/19, 13:00	05/09/19, 05:15
	On	05/09/19, 05:15	06/09/19, 08:30

Table S2. Evolution of isotopic and elementary composition of O_2 for the empty chamber test (starting 21 October 2020).

Date	$\delta^{18}O$ of O_2 (‰)	$\Delta O_2/N_2$ (‰)
21 October 2020	-0.055	-3.075
29 October 2020	-0.046	-3.628
06 November 2020	-0.051	-6.218
17 November 2020	-0.064	-8.8

Table S3. Oxygen isotopic ratios for leaf water (lw), irrigation water (iw) and soil water (sw) at the beginning (t0) and end of the sequence (tf) of the photosynthesis and dark respiration experiment. The ${}^{17}R$ values are calculated here with a value of 12.03 ‰ (Luz and Barkan, 2011) for determination of the $\delta^{17}O$ of atmospheric O_2 vs $\delta^{17}O$ of VSMOW.

Sequence	${}^{18}R_{lw}$	${}^{17}R_{lw}$	${}^{18}R_{iw,t0}$	${}^{17}R_{iw,t0}$	${}^{18}R_{sw,tf}$	${}^{17}R_{sw,tf}$
1	0.9802	0.9899	0.9712	0.9852	0.9723	0.9858
2	0.9776	0.9885	0.9712	0.9852	0.9722	0.9857
3	0.9763	0.9878	0.9712	0.9852	0.9726	0.9859

Section S1: Sensitivity tests to the flux of dark leaf respiration during the day

The rate of autotrophic respiration (dark leaf respiration) is expected to be lower during light periods than during dark periods (Tcherkez et al., 2017) which was not considered in the main text. These sensitivity tests hence aim at quantifying how the value of $\alpha_{photosynthesis}$ is affected when F_{dark_respi} changes from a maximum value (F_{dark_respi} during dark period) to an extreme minimum value (F_{soil_respi} during dark period, hence no dark leaf respiration during the light period) and when α_{dark_respi} changes from the global value α_{dark_respi} including leaf and soil respiration as during dark period to the value α_{soil_respi} measured during dark period. We test as well the combined effect of modification of both F_{dark_respi} and α_{dark_respi}. The results from these sensitivity tests (Table S4) show variations in $\alpha_{photosynthesis}$ within a range which is smaller than the analytical uncertainty range found for our initial determination of $\alpha_{photosynthesis}$. In particular, we found that when we modify both F_{dark_respi} and α_{dark_respi} to consider the extreme situation with only soil respiration, the mean value of ${}^{18}\alpha_{photosynthesis}$ is unchanged.

Table S4. $\alpha_{photosynthesis}$ values obtained from sensitivity tests with respect to different flux and fractionation factors associated with dark respiration during the day. Subscript 0: fractionation factor and flux for dark respiration during the day are the same as those determined during the night (case described in the main text). Subscript 1: flux of dark respiration during the day is taken equal to the flux of soil respiration (no flux of dark leaf respiration), fractionation factor for dark respiration during the day is the same as during the night. Subscript 2: flux of dark respiration during the day is the same as during the night, fractionation factor for dark respiration during the day is equal to α_{soil_respi}. Subscript 3: flux of dark respiration during the day is taken equal to the flux of soil respiration, fractionation factor for dark respiration during the day is equal to α_{soil_respi}. μ is the average over all lines above of the different quantities and σ the associated standard deviation.

Sequence	Period	${}^{18}\alpha_{photosynthesis,0}$	${}^{18}\alpha_{photosynthesis,1}$	${}^{18}\alpha_{photosynthesis,2}$	${}^{18}\alpha_{photosynthesis,3}$	${}^{17}\alpha_{photosynthesis,0}$	${}^{17}\alpha_{photosynthesis,1}$	${}^{17}\alpha_{photosynthesis,2}$	${}^{17}\alpha_{photosynthesis,3}$
1	1	0.9947	0.9931	0.9948	0.9933	0.9972	0.9964	0.9972	0.9965
	2	1.0038	1.0038	1.0039	1.0038	1.0019	1.0019	1.0020	1.0019
	3	1.0037	1.0036	1.0038	1.0036	1.0016	1.0016	1.0017	1.0016
2	1	1.0023	1.0023	1.0033	1.0023	1.0024	1.0011	1.0017	1.0012
	2	1.0043	1.0046	1.0051	1.0046	1.0043	1.0020	1.0023	1.0021
3	1	1.0039	1.0032	1.0047	1.0039	1.0020	1.0017	1.0024	1.0018
	2	1.0024	1.0010	1.0033	1.0021	1.0014	1.0008	1.0019	1.0010
	3	1.0060	1.0059	1.0074	1.0068	1.0032	1.0031	1.0038	1.0034
μ		1.0026	1.0022	1.0033	1.0026	1.0018	1.0011	1.0016	1.0012
σ		0.0034	0.0039	0.0037	0.0040	0.0021	0.0020	0.0019	0.0020

Table S5. Complete data set of the different fractionation factors (${}^{18}\alpha_{soil\_respi}$) and (${}^{17}\alpha_{soil\_respi}$) and isotopic discriminations (${}^{18}\varepsilon_{soil\_respi}$ and ${}^{17}\varepsilon_{soil\_respi}$) of dioxygen and respiratory fluxes during soil respiration experiment. μ is the average over all lines above for the different quantities and σ the associated standard deviation.

Sequence	${}^{18}\alpha_{soil\_respi}$	${}^{18}\varepsilon_{soil\_respi}$ (‰)	${}^{17}\alpha_{soil\_respi}$	${}^{17}\varepsilon_{soil\_respi}$(‰)	θ_{soil_respi}	O_2 consumed ($mmolO_2$/day)
1	0.9883	-11.7	0.9940	-6.0	0.5170	2.81
2	0.9861	-13.9	0.9928	-7.2	0.5157	11.78
3	0.9898	-10.2	0.9947	-5.3	0.5163	8.92
4	0.9866	-13.4	0.9930	-6.9	0.5165	6.59
μ	0.9877	-12.3	0.9936	-6.4	0.5164	7.52
σ	0.0017	1.7	0.0009	0.9	0.0005	3.79

Table S6. Complete data set of the different fractionation factors (${}^{18}\alpha_{dark\_respi}$) and (${}^{17}\alpha_{dark\_respi}$) of dioxygen during dark periods of the photosynthesis and respiration experiment. μ is the average over all lines above for the different quantities and σ the associated standard deviation. The missing data are due to a problem during measurements.

Sequence	Period	${}^{18}\alpha_{dark\_respi}$	${}^{17}\alpha_{dark\_respi}$	θ_{dark_respi}	O_2 consumed ($mmolO_2$/day)
1	1	0.9846	0.9921	0.5105	21.77
	2	0.9844	0.9921	0.5045	21.46
	3	0.9848	0.9922	0.5084	18.57
	4	0.9841	0.9917	0.5224	14.98
2	1	0.9822	0.9909	0.5077	36.61
	2	0.9794			25.93
	3	0.9825	0.9909	0.5183	31.03
3	1	0.9821	0.9911	0.4977	26.17
	2	0.9803	0.9897	0.5238	26.15
	3	0.9853	0.9924	0.5141	31.65
μ		0.9830	0.9914	0.5124	25.43
σ		0.0020	0.0008	0.0084	6.51

Section S2: Sensitivity tests to the fractions and fractionation factors of the different biological dioxygen uptakes

We quantify how much the uncertainties in the fractions of the biological process (dark respiration, photorespiration and the Mehler reaction) could affect the values of dark respiratory and photosynthetic fractionation factors determined in our study (Tables S7, S8 and S9). For this, we start from the values given by Landais et al. (2007): f_{dark_respi} (0.6), $f_{photorespi}$ (0.3) and f_{Mehler} (0.1) and varied the fractions as indicated in Table S5 (tests 0, 1 and 2).

In addition, we did other tests for uncertainties in the values of the fractionation factor of photorespiration given by Helman et al. (2005) (test 3 in grey columns of Tables S5, S6 and S7). The initial values for photorespiration isotopic discrimination are respectively - 21.3 ‰ and - 11.07 ‰ for $^{18}\varepsilon_{photo\_respi}$ and $^{17}\varepsilon_{photo\_respi}$ as given by Helman et al. (2005). From uncertainties on these values of 0.5 ‰ and 0.3 ‰ for $^{18}\varepsilon_{photo\_respi}$ and $^{17}\varepsilon_{photo\_respi}$ (Helman et al., 2005), we used alternative values of -20.8 ‰ and -11.04 ‰ for $^{18}\varepsilon_{photo\_respi}$ and $^{17}\varepsilon_{photo\_respi}$. For the Mehler reaction we also chose the value proposed by Helman et al. (2005): $^{18}\varepsilon_{Mehler} =$ - 10.8 ‰ and $^{17}\varepsilon_{Mehler} =$ - 5.7 ‰. We also tested the uncertainty in the fractionation factors of the Mehler reaction (test 4) as given by Guy et al. (1993): $^{18}\varepsilon_{Mehler} =$ - 15.3 ‰ and $^{17}\varepsilon_{Mehler} =$ - 7.98 ‰.

Table S7. Details of input parameters for sensitivity tests. White columns detail the tests on the fractions of oxygen consumption; grey columns detail the test on the values of isotopic discrimination.

	Test 0	Test 1	Test 2	Test 3	Test 4
f_{dark_respi}	1	0.9	0.4	0.6	0.6
f_{photo_respi}	0	0	0.5	0.3	0.3
f_{Mehler}	0	0.1	0.1	0.1	0.1
$^{18}\varepsilon_{photo\_respi}$	-21.3	-21.3	-21.3	-20.8	-20.8
$^{17}\varepsilon_{photo\_respi}$	-11.07	-11.07	-11.07	-11.04	-11.04
$^{18}\varepsilon_{Mehler}$	-10.8	-10.8	-10.8	-10.8	-15.3
$^{17}\varepsilon_{Mehler}$	-5.7	-5.7	-5.7	-5.7	-7.9

Table S8. α_{total_respi} values obtained from the sensitivity tests: results of sensitivity tests on dioxygen consumption fractions (white columns) and on uncertainties in fractionation factor values (grey columns). μ is the average over all lines above of the different quantities and σ the associated standard deviation. The missing data are due to a problem during measurements.

Sequence	Period	$^{18}\alpha_{total\_res}$ Test 0	$^{18}\alpha_{total\_res}$ Test 1	$^{18}\alpha_{total\_res}$ Test 2	$^{18}\alpha_{total\_res}$ Test 3	$^{18}\alpha_{total\_resp}$ Test 4	$^{17}\alpha_{total\_res}$ Test 0	$^{17}\alpha_{total\_res}$ Test 1	$^{17}\alpha_{total\_res}$ Test 2	$^{17}\alpha_{total\_res}$ Test 3	$^{17}\alpha_{total\_resp}$ Test 4
1	1	0.9868	0.9870	0.9830	0.9847	0.9841	0.9933	0.9934	0.9912	0.9921	0.9932
	2	0.9865	0.9868	0.9829	0.9846	0.9840	0.9933	0.9934	0.9912	0.9921	0.9932
	3	0.9871	0.9873	0.9831	0.9849	0.9843	0.9935	0.9936	0.9913	09922	0.9934
	4	0.9859	0.9862	0.9826	0.9842	0.9836	0.9926	0.9927	0.9909	0.9917	0.9925
2	1	0.9828	0.9835	0.9814	0.9824	0.9818	0.9913	0.9916	0.9904	0.9909	0.9906
	2	0.9781	0.9792	0.9795	0.9795	0.9789					
	3	0.9834	0.9839	0.9816	0.9827	0.9820	0.9913	0.9916	0.9904	0.9909	0.9907
3	1	0.9827	0.9833	0.9813	0.9822	0.9908	0.9916	0.9919	0.9908	0.9911	1.0020
	2	0.9797	0.9806	0.9801	0.9805	0.9894	0.9892	0.9897	0.9898	0.9897	1.0014
	3	0.9879	0.9880	0.9834	0.9854	0.9922	0.9938	0.9939	0.9917	0.9924	1.0031
μ		0.9838	0.9843	0.9818	0.9829	0.9861	0.9922	0.9924	0.9909	0.9914	0.9918
σ		0.0031	0.0028	0.0013	0.0019	0.0061	0.0014	0.0013	0.00055	0.0008	0.0014

Table S9. $\alpha_{photosynthesis}$ values obtained from the sensitivity tests: results of sensitivity tests on dioxygen consumption fractions (white columns) and on uncertainties in fractionation factor values (grey columns). μ is the average over all lines above of the different quantities and σ the associated standard deviation.

Sequence	Period	$^{18}\alpha_{photosynthe}$ Test 0	$^{18}\alpha_{photosynthe}$ Test 1	$^{18}\alpha_{photosynthe}$ Test 2	$^{18}\alpha_{photosynthe}$ Test 3	$^{18}\alpha_{photosynthesi}$ Test 4	$^{17}\alpha_{photosynthe}$ Test 0	$^{17}\alpha_{photosynth}$ Test 1	$^{17}\alpha_{photosynth}$ Test 2	$^{17}\alpha_{photosynth}$ Test 3	$^{17}\alpha_{photosynthesis4}$ Test 4
1	1	0.9941	0.9944	0.9951	0.9969	0.9974	0.9969	0.9970	0.9974	0.9972	0.9974
	2	1.0040	1.0040	1.0036	1.0038	1.0021	1.0020	1.0020	1.0018	1.0019	1.0020
	3	1.0039	1.0039	1.0034	1.0037	1.0018	1.0017	1.0017	1.0015	1.0016	1.0018
2	1	1.0021	1.0023	1.0023	1.0024	1.0022	1.0013	1.0014	1.0013	1.0013	1.0022
	2	1.0043	1.0044	1.0040	1.0043	1.0042	1.0021	1.0021	1.0019	1.0020	1.0042
3	1	1.0037	1.0039	1.0039	1.0040	1.0038	1.0096	1.0021	1.0021	1.0021	1.0020
	2	1.0019	1.0022	1.0026	1.0024	1.0022	1.0013	1.0014	1.0016	1.0014	1.0014
	3	1.0063	1.0066	1.0056	1.0062	1.0059	1.0034	1.0035	1.0030	1.0035	1.0031
μ		1.0026	1.0027	1.0026	1.0030	1.0025	1.0023	1.0014	1.0013	1.0013	1.0014
σ		0.0039	0.0034	0.0030	0.0025	0.0034	0.0033	0.0018	0.0016	0.0017	0.0017

Table S10. Complete data set of the different average photosynthesis coefficient fractionations of oxygen during light periods during the photosynthesis and respiration experiment. μ is the average over all lines above of the different quantities and σ the associated standard deviation.

Sequence	Period	$^{18}\alpha_{photosynthesis}$	$^{17}\alpha_{photosynthesis}$	$\theta_{photosynthesis}$
1	1	0.9947	0.9972	0.5296
	2	1.0038	1.0019	0.5067
	3	1.0037	1.0016	0.4420
2	1	1.0023	1.0013	0.5672
	2	1.0043	1.0020	0.4794
3	1	1.0039	1.0020	0.5202
	2	1.0024	1.0014	0.6047
	3	1.0061	1.0032	0.5248
μ		1.0027	1.0013	0.5218
σ		0.0032	0.0017	0.0498

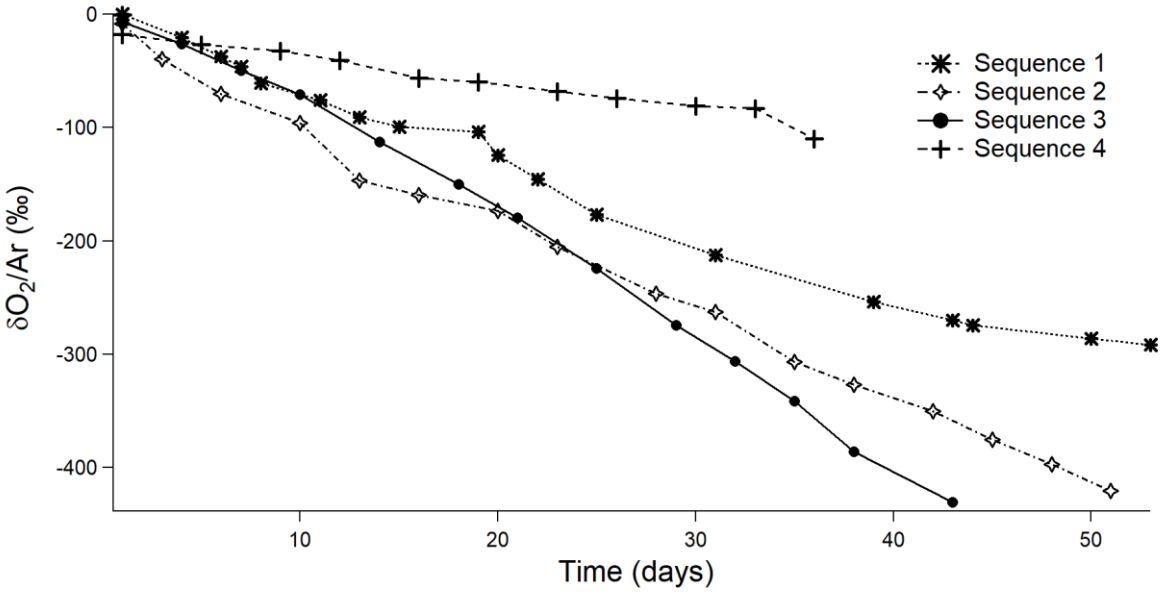
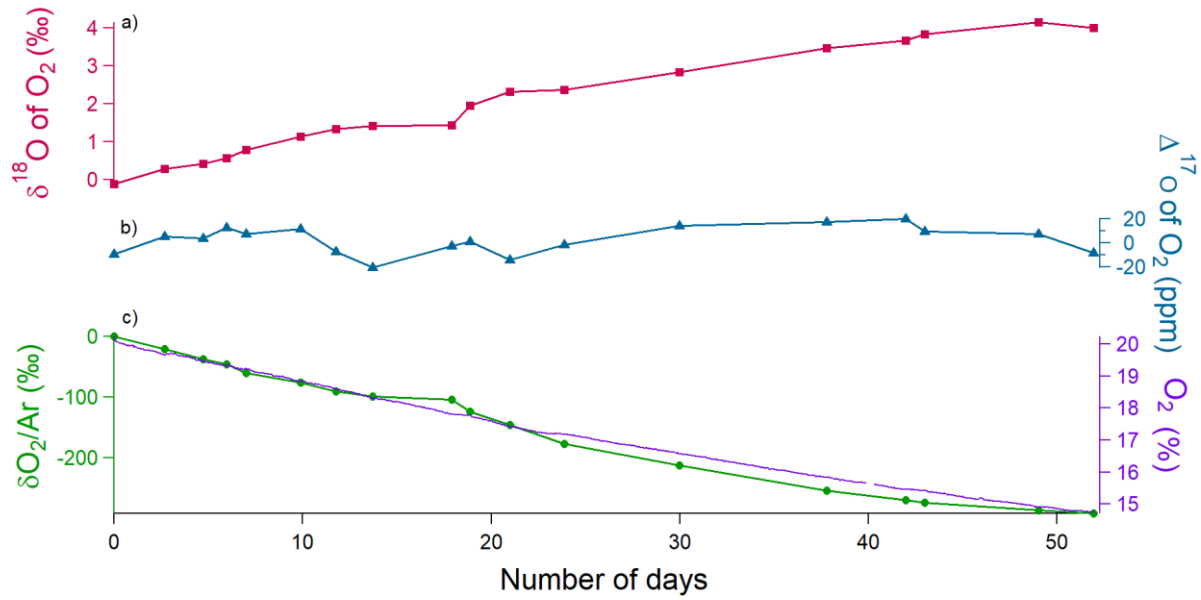
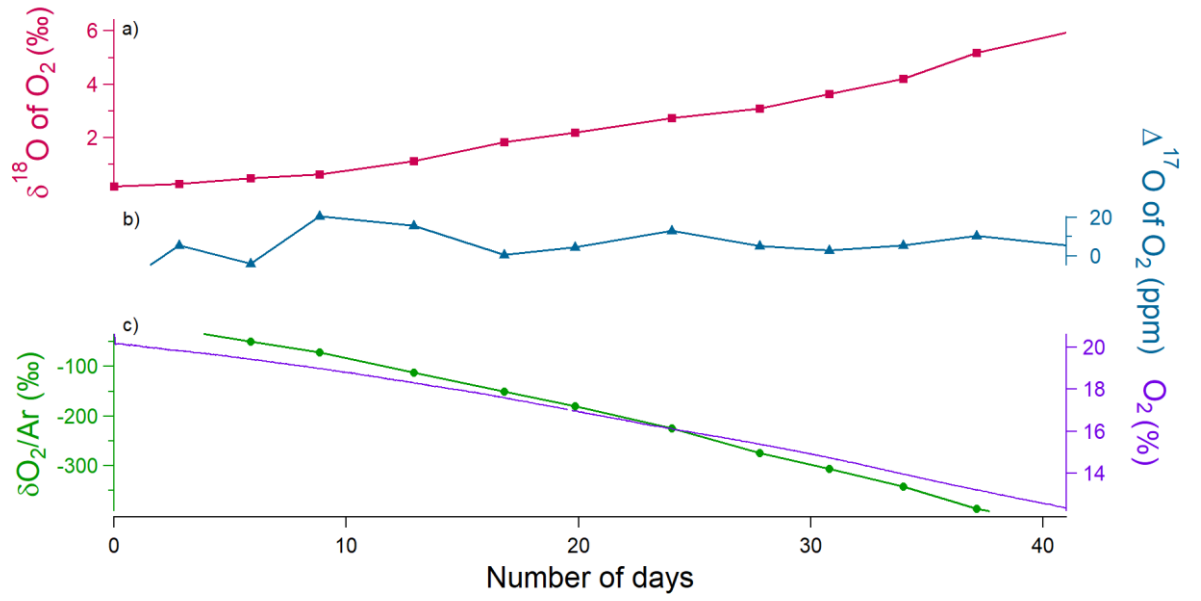


Fig.S1. Comparison of the evolution of the O_2 concentration of the sequence 1, 2, 3 and 4 during the soil respiration experiment.

1)


a)
b)
c)
δ18O of O2 (‰)
Δ 17O of O2 (ppm)
δO2/Ar (‰)
O2 (%)
Number of days

2)


a)
b)
c)
δ18O of O2 (‰)
Δ 17O of O2 (ppm)
δO2/Ar (‰)
O2 (%)
Number of days

3)

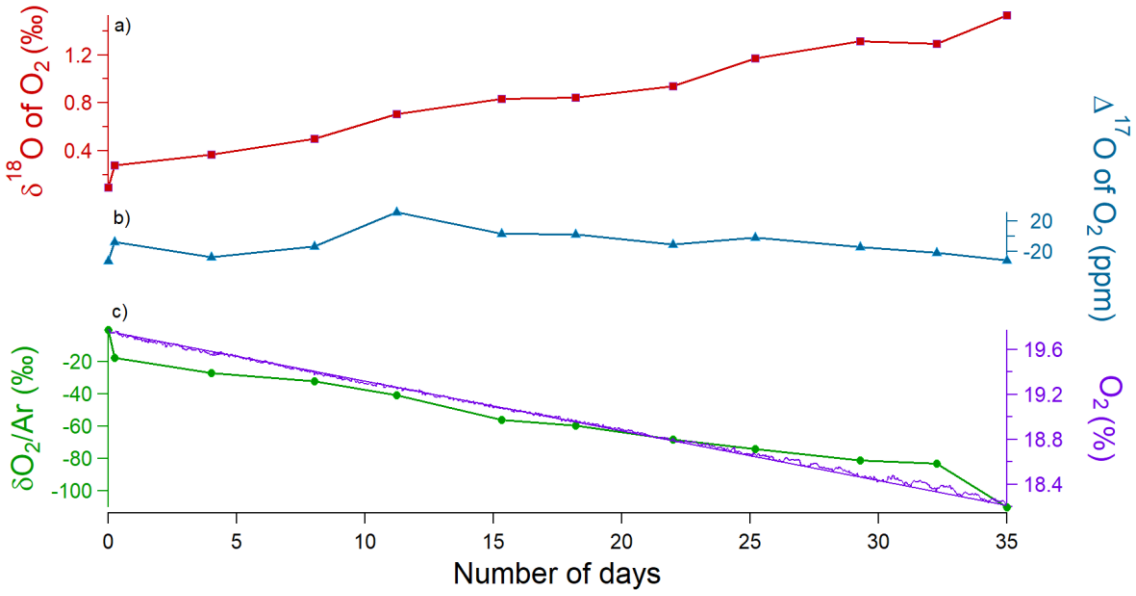
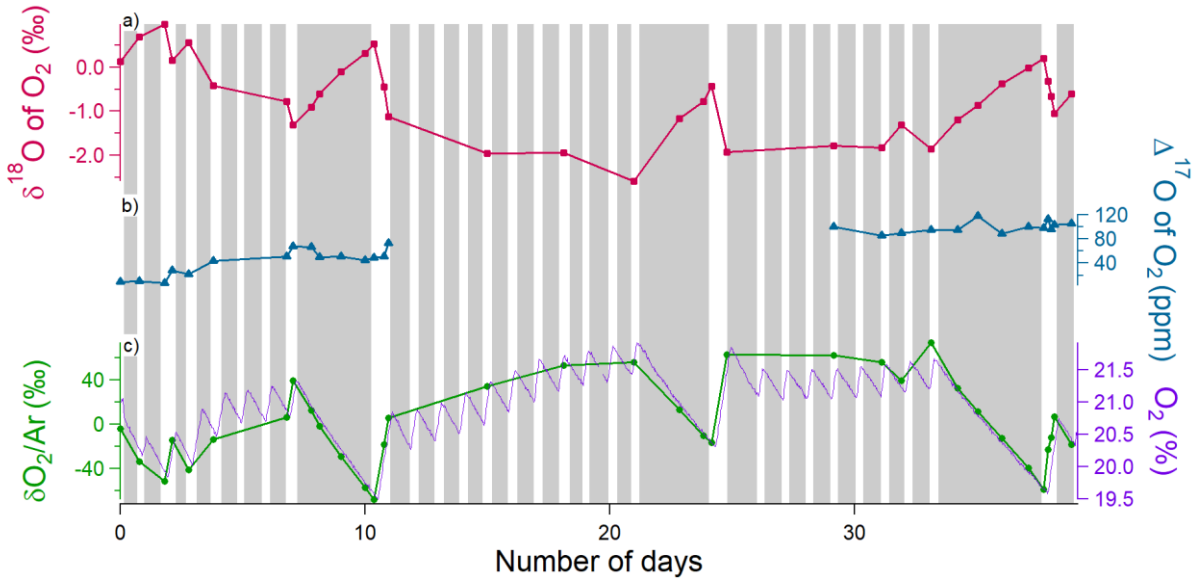


Fig.S2. Summary of the first (1), third (2) and fourth (3) sequences of the soil respiration experiment (day 0 is the beginning of the sequence). (a) $\delta^{18}O$ of O_2 (red) variations. (b) $\Delta^{17}O$ of O_2 (blue) variations. (c) Dioxygen concentration (purple) from the optical sensor and $\delta O_2/Ar$ variations (green) measured by IRMS.

1)



2)

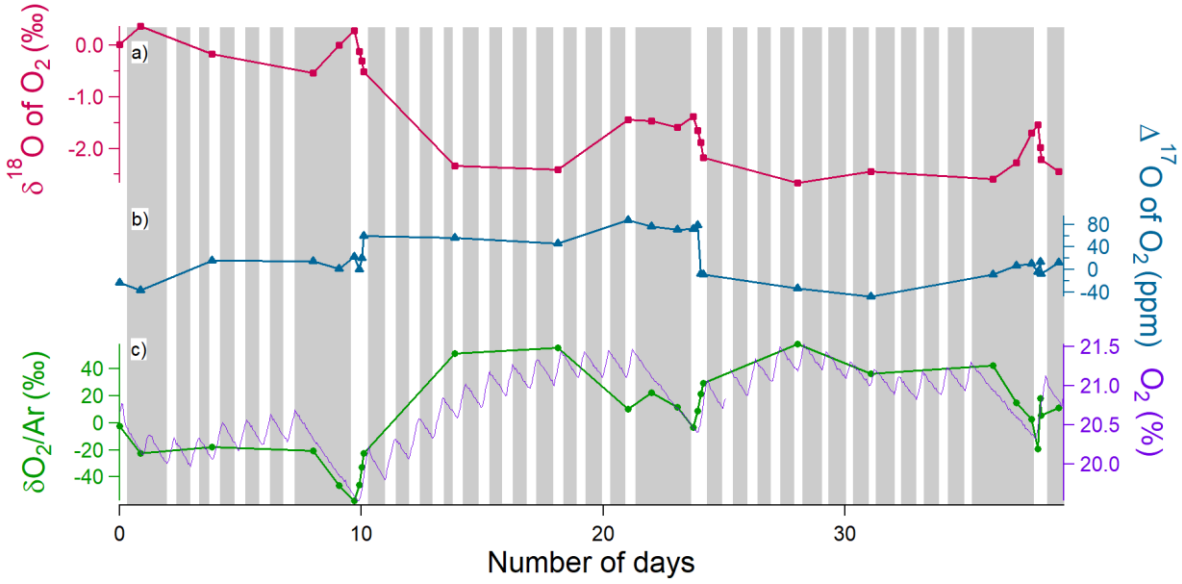


Fig.S3. Summary of the second (1) and third (2) sequences of the photosynthesis and dark respiration experiment (day 0 is the beginning of the sequence). Grey rectangles correspond to night periods and white rectangles to light periods. (a) $\delta^{18}O$ of O_2 (red) variations. (b) $\Delta^{17}O$ of O_2 (blue) variations. (c) Dioxygen concentration (purple) from the optical sensor and $\delta O_2/Ar$ variations (green) measured by IRMS.