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ABSTRACT

We report the release of oxygen (O_2) under dark conditions in aerobic soils. This unexpected process is hidden by respiration which constitutes the dominating reversal O_2 flux. By using $H_2{}^{18}O$ in different soils, we confirmed that $^{16}O^{18}O$ and $^{18}O_2$ released under dark soil conditions originated from added $H_2{}^{18}O$. Water is the only large-scale source of electrons for reduction of CO_2 in soils, but it has not been considered as an electron donor because of the very strong oxidation system needed. A high share of soil inorganic material seems to favor the release of O_2.

Keywords: dark water oxidation, soil CO_2 reduction, soil oxygen release, soil respiration

To access the supplementary material to this article, please see Supplementary files under Article Tools online.

1. Introduction

1.1. Background

Oxidation of water leading to the release of oxygen (O_2) has been explicitly associated with photosynthetic organisms. This process was studied by Robert Hill more than 70 yr ago (Walker, 2002). No other known large-scale aerobic biological O_2 production has been discovered. This is understandable since the O_2-evolving complex is one of the strongest biological oxidation systems known (Wydrzynski, 2008). Under anaerobic conditions, O_2 production has been shown by chlorate-reducing bacteria (Nilsson et al., 2013), and denitrification with methane as an electron donor has been associated with the release of O_2 as an intermediate (Ettwig et al., 2010, 2012).

1.2. Initial observations

Previous studies indicated that a part of CO_2 in the soil atmosphere was allocated into an internal soil CO_2 sink in different types of soils (Fleischer and Bouse, 2008; Fleischer, 2012). The process occurred in darkness, that is, photosynthesis could not explain this within-soil reduction of CO_2. The mechanism of this process remains speculative; however, the supply of NH_4^+ stimulated the use of CO_2 as a carbon source thus indicating that nitrification was the possible chemoautotrophic process involved. Therefore, consumption of O_2 was expected to increase as a result of the O_2-consuming NH_4^+ additions, but instead it decreased. This might be interpreted as within-soil release of O_2. A deeper insight into the prerequisites for these findings was required, and this study was initiated.

1.3. Hypothesis

If we suppose that a chemoautotrophic process in soils drives the reduction of CO_2, we must answer an important question: Where do the electrons come from? The only source available on a large scale is water. However, the possibility that soil nitrification drives the reduction of CO_2 using H_2O as an electron donor does not coincide with our current knowledge. This implies that the release of O_2 is masked by the predominating O_2 consumption in soils.

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If oxidation of water, in some way analogous to the process in green plants, occurred in the soil darkness, would tests using $H_2{}^{18}O$ demonstrate a release of $^{18}O_2$?

2. Materials and methods

We used sieved (2 mm) composite soil samples (ø25 mm) from 0 to 5 cm depth. The fresh litter of the O-horizon was excluded. The samples were transported at near sampling temperatures (approx. $\pm 2^{\circ}C$ deviation) and safely protected from light from the day of sampling until they were analysed.

For incubations with added $H_2{}^{18}O$, we used 12-ml Exetainers containing 0.5 or 1 g soil. The Exetainers were initially open to air for 3-11 d at incubation temperature. High moisture samples took the longest time. This enabled loss of moisture later compensated for again by the additions at incubation start. Oscillating moisture is the natural situation in soils following precipitation/dry periods. In addition to being kept in the darkness, the Exetainers were also wrapped with aluminum foil as an additional safety measure.

$H_2{}^{18}O$ (97% ^{18}O) was from Armar Chemicals in Döttingen, Switzerland. At the start of incubation, 100 or 150 µl $H_2{}^{18}O$ was added. One hundred microlitres of formalin (4%) per gram of soil did not inhibit all biological activity in one sample initially tested. Double dose (200 µl) of formalin was therefore used, in addition to one sample with 100 µl of a strong $HgCl_2$ water solution (15 mg ml^{-1}), which was also used as a second, parallel control that is very efficient under the prevailing aerobic conditions (Trevors, 1996). Microbial activity was inhibited in all samples that received formalin or $HgCl_2$. The results are based on three incubations (the two references, and the non-poisoned sample from which microbial respiration and release of $^{18}O_2$ and $^{16}O^{18}O$ was calculated).

The amount of water added was the same in the intact and inhibited samples. The Exetainers were then sealed. Samples from 2011 were incubated for longer periods (Tables 2 and 3) until measurement of the isotopic composition in the atmosphere of the Exetainers.

An OmniStar™ quadrupole gas mass spectrometer, Pfeiffer Vacuum, was used to determine the oxygen gas masses; 32 u, 34 u and 36 u, and the carbon dioxide mass 44 u. A sample needle attached to the gas-MS was used to penetrate the septum of the sealed Exetainer. The gas-MS was operated in *multiple ion detection* mode collecting data to a single file for all measurements within a dataset to ensure as stable an operation of the ion emission source, quadrupole system and detector as possible.

The data section assigned to each sample started once a visibly stable signal was achieved from the instrument, normally 1–2 min after septum penetration. For each sample, data were recorded for around 2 min (approx. 2 sec/point), whereof the last 30 data points were used to determine an average signal current and its standard deviation for respective mass of interest. Before each sample, a measurement on ambient air was used to recalibrate the system and check for the gas-MS stability. Further details on the method are found in Supplementary file.

All values recorded in the data file were corrected for the gas-MS vacuum chamber pressure fluctuations at a single data point level. The air measurement preceding each sample was used to determine the average background signal and its standard deviation over 100 data points. The average $BG + 3 \cdot SD_{BG}$ was used as a limit of qualitative determination, and the average $BG + 10 \cdot SD_{BG}$ was used as a limit of quantitative determination (Currie, 1968); see Supplementary file.

Ordinary air was used as the start atmosphere in all Exetainers. As a result, a subtraction of the preceding air measurement automatically corrects the measurement for naturally occurring species at respective mass of interest. In this case, mainly the correction of mass 36 for its ^{36}Ar contents is useful in order not to overestimate the $^{18}O_2$ production in the experiments. A new calibration was made for each sample based on the air measurement preceding it; details on the calibration procedure are found in Supplementary file. A complete calculation from measured current to tabulated O_2 production rates is given for the Biskopstorp sample in Supplementary file.

Sampling sites are described in Table 1. After 2 h, the pH of 10 g soil in 25 ml of water/1 M KCl was measured.

3. Results

During an initial study in 2009, 14 soil samples were collected from 11 Swedish and German forest, grassland and agricultural sites. Oxygen in CO_2 can have a respiratory origin, but can also be the result of abiotic water–carbonic acid exchange. The CO_2 molecular weights 46 and 48, as well as 44 originating from the water in the fresh soil sample, were found. Significant production of $^{18}O_2$ and $^{18}O^{16}O$ was detected in 10 of the 14 soil samples (Table 2).

Whether O_2 released to the soil atmosphere was the result of a biological process deserves to be unequivocally delineated. We therefore used controls with inhibited biological activity during the tracer study in 2011. In the inhibited controls, no release of $^{18}O_2$ or $^{18}O^{16}O$ occurred. Release of $^{18}O_2$ and $^{18}O^{16}O$ was associated with uninhibited soil, but it was low in the acidic soils (Table 3). At the two sites Annaberg and Sparneck, with very acidic soils, no release of $^{18}O_2$ or $^{18}O^{16}O$ was detected and soil respiration was low.

Table 1. Characteristics of the sites studied.

Site	Longitude, latitude	General observations and soil characteristics	pH KCl	pH H_2O	LOI percent of dry matter	Additional site characteristics comments
Biskopstorp Nature reserve SW Sweden	56°47′N, 12°53′E	300-year-old beech forest, silty loam brown earth	2.5	3.9	56.5	
Tolarp SW Sweden	56°41′N, 13°00′E	Birch, rich in herbs, loamy sand	4.0	5.4	12.0	
Skällås Tönnersjöheden forest research station SW Sweden	56°42′N, 13°07′E	Norway spruce, fertilised with 30 kg P and 60 kg K ha^{-1} every 7th year sandy–silty till	2.9	3.8	27.5	Sikström, 2005
Mellby agricultural research fields, SW Sweden	56°28′N, 12°59′E	Unfertilised grassland loamy sand	4.7	5.9	5.55	
Mellby agricultural research fields, plot I	56°28′N, 12°59′E	Crop rotation, no catch crop loamy sand	4.9	6.3	5.95	Liu et al., 2012
Mellby agricultural research fields, plot J	56°28′N, 12°59′E	Crop rotation, catch crop till 2006 loamy sand	4.9	6.3	6.31	Liu et al., 2012
Mellby agricultural research fields, plot G	56°28′N, 12°59′E	Crop rotation, no catch crop loamy sand	4.8	6.0	5.74	Liu et al., 2012
Mellby agricultural research fields, plot H	56°28′N, 12°59′E	Crop rotation, catch crop loamy sand	5.1	6.1	6.01	Liu et al., 2012
Annaberg Saxony, Germany	50°33′N, 12°58′E	Spruce Wind-exposed forest edge, silt	2.7	3.5	50.6	Remains of mining in spots
Tannenberg Saxony, Germany	50°36′N, 12°56′E	Spruce Closed forest, silt	3.1	4.1	27.8	The forest has been limed
Grillenburg Saxony, Germany	50°57′N, 13°30′E	Spruce 10 m from forest edge, podsol-brown earths, loam	2.7	3.3	64.2	Nearby area described by Schwärzel et al., 2009
Hainich National Park Thuringia, Germany	51°04′N, 10°27′E	Closed deciduous forest dominated by old beech clayey brown earth	4.4	5.4	18.0	Knohl et al., 2003 Klaus and Stephan, 1998
Sparneck, Fichtelgebirge, sampling at 2, 30 and 50 m from forest edge, Bavaria, Germany	50°09′N, 11°51′E	Spruce at a 5 m broad forest edge, closed forest clearcut, with spotted lingering surface water clay loam	2.5	3.3	81.0 (edge) 66.0 (30 m) 75.5 (50 m)	Nearby area described by Gerstberger, 2001

Soil samples are from 0 to 5 cm. LOI (loss on ignition) is organic matter.

4. Discussion and conclusion

The most obvious risk of misdetection due to other elements or compounds with a mass of 34 or 36 is that of ^{36}S, but this would require that elemental sulphur in the gas phase, which is highly unlikely. A small fraction of ^{36}Ar occurs naturally which we corrected for, using background subtraction. Hydrochloric acid (pK_a −7) is not a constituent of the soils and the release of this compound, if present in soils with pH > 3, would require it to be carried over to the gas phase, which is also unlikely. The highest release of $^{18}O_2$ and $^{18}O^{16}O$ occurred in soils with pH > 5. ^{35}Cl and ^{37}Cl amounts to 76 and 24% naturally, but no hydrochloric acid could be detected at mass 38, that is, there is no HCl interference at mass 36. These deductions are further supported by those analyses that indicated nothing but background at mass 36 in some of the samples. This implies that not only was $^{18}O_2$ not found, but possible misdetections of the other elements/compounds can be excluded. Moreover, masses 36 and 34, over background levels, were only detected in the uninhibited samples. If the process is solely non-biological, there should be at least traces above the background of $^{18}O_2$ present in inhibited samples. Since this is not the case, $^{18}O_2$ was only detected in biologically active samples, the most probable explanation is that a biological process is the source of $^{18}O_2$.

Some biological processes, which can result in O_2 production in the dark, are known. Most well known is the decomposition of hydrogen peroxide mostly attributed to ultraviolet radiation in aquatic environments (Pamatmat, 1997; Wilson et al., 2000). If occurring in the dark, $H_2{}^{18}O_2$ is formed from the reduction of $^{18}O_2$ which, in turn, must first be released by the indicated oxidation of $H_2{}^{18}O$.

Table 2. Release of $^{18}O_2$ and $^{18}O^{16}O$ after dark incubation of $H_2{}^{18}O$ in different soils during autumn 2009

Site	Sampling date 2009	Incubation (days)	$^{18}O_2$ [μg/(g_{LOI}·d)]	$^{18}O^{16}O$ [μg/(g_{LOI}·d)]
Biskopstorp	24 Sept	5	n.d.	n.d.
	20 Nov	21	n.d.	n.d.
Tolarp	26 Sept	4	0.55	2.0
Mellby grassland	28 Sept	3	6.3	19
Mellby plot J	28 Sept	3	1.3	3.6
	27 Nov	17	+	n.d.
Mellby plot I	28 Sept	3	2.6	9
	27 Nov	17	+	n.d
Annaberg	11 Oct	27	n.d.	n.d.
Tannenberg	11 Oct	27	0.02	0.02
Grillenburg	12 Oct	27	n.d.	n.d
Mellby plot H	15 Oct	27	3.1	8.5
Mellby plot G	15 Oct	27	2.3	5.6
Skällås	11 Nov	5	4.0	11.4

+, detected, not quantified; n.d., not detected.
LOI (loss on ignition) refers to the fresh soil sample at the sampling occasion. Additional site information is given in Table 1.

But $H_2{}^{18}O$ was added to soil that was constantly kept dark. A direct hydrogen peroxide pathway to $^{18}O_2$ is not feasible under the experimental conditions described above.

A second biological process that leads to O_2 production is microbial oxochlorate respiration (Nilsson et al., 2013), in which oxygen is produced when chlorite is decomposed. However, it is unlikely that the soil samples used in this study contain significant amounts of oxochlorates. Oxochlorates only accumulate to measureable amounts when leaching and microbial activity is low, for example, in very arid environments. Therefore, we think that this alternative can be ruled out.

Finally, it has been suggested that oxygen is produced by dismutation of nitric oxide (NO) in anaerobic methane

Table 3. Release of $^{18}O_2$ and $^{18}O^{16}O$, and simultaneous soil respiration estimated as decrease in $^{16}O_2$ after dark incubation of $H_2{}^{18}O$ in different soils during the summer 2011

Site	Sampling date	Incubation (days)	$^{16}O_2$ cons. [μg/(g_{LOI}·d)]	$^{18}O_2$ prod. [μg/(g_{LOI}·d)]	$^{16,\ 18}O_2$ prod. [μg/(g_{LOI}·d)]
Hainich	13 June	41	140	0.33	4.0
Annaberg	14 June	41	70	n.d.	n.d.
Tannenberg	14 June	41	100	0.07	0.48
	17 Sept	18	520	0.20	2.2
Grillenburg	15 June	41	97	n.d.	n.d.
	17 Sept	18	570	0.07	n.d.
Skällås	26 June	41	189	n.d.	n.d.
	6 Sept	28	504	n.d.	n.d.
Tolarp	28 June	41	415	0.33	2.7
	8 July	28	342	0.14	1.9
	25 July	14	516	0.09	+
Mellby grassland	4 Sept	28	626	3.4	25
	26 Sept	14	897	2.1	7.3
Mellby H	4 Sept	28	+	1.5	11
Biskopstorp	9 Sept	28	369	0.07	n.d.
Mellby G	10 Sept	28	333	1.9	15
	26 Sept	14	381	3.7	14
Sparneck					
2 m	19 Sept	18	65	n.d.	n.d.
30 m	19 Sept	18	+	n.d.	n.d.
50 m	19 Sept	18	+	n.d.	n.d.

+, detected, not quantified; n.d., not detected.
LOI (loss on ignition) refers to the fresh soil sample at the sampling occasion. Additional site information is given in Table 1.

oxidisers (Ettwig et al., 2010, 2012). These organisms appear to utilise an alternative mode of denitrification, involving disproportionation of NO into N_2 and O_2 instead of reduction to N_2O. The O_2 produced appears to be used for oxidation of reduced substrates, for example, methane. As nitrate and nitrite are present in the soil samples used, we cannot exclude the possibility that an NO dismutase is involved in oxygen production in our experiments. However, this would require that denitrification processes (including the NO dismutase) are active in the soil samples under the strictly aerobic conditions in our study.

Non-biological production of O_2 that requires large amounts of energy at high pressures or at very high temperatures, including thermal decomposition of water, and electrolysis, are known technologies that can be disregarded since the incubations were carried out at 15°C and atmospheric pressure.

A largely unexpected biochemical pathway in forest, agricultural and grassland soils is indicated. It is masked by the reversal, dominating soil respiration concurrently consuming O_2 in the soil. After 41 d of $H_2{}^{18}O$-incubation in darkness the relation of $^{18}O^{16}O$ to $^{18}O_2$ was still high in some of the samples, that is, the share of $H_2{}^{16}O$ from the original fresh sample was high. In addition to the role of pH, one clue from our results could be that the most active O_2 releasing organic material was in soils with a high share of inorganic material (Fig. 1) which, similar to sorption of organic material (Kalbitz et al., 2005), provides sufficient facilities for biofilm attachments to mineral surfaces. As has been reported, nitrifiers are known to develop protecting biofilms (Hagopian and Riley, 1998). However, recent indications of almost ubiquitous nitrification including Archaea and Bacteria (Martens-Habbena et al., 2009) call for a more in-depth study to identify the ecological niches with dark oxidation of water in aerobic soils.

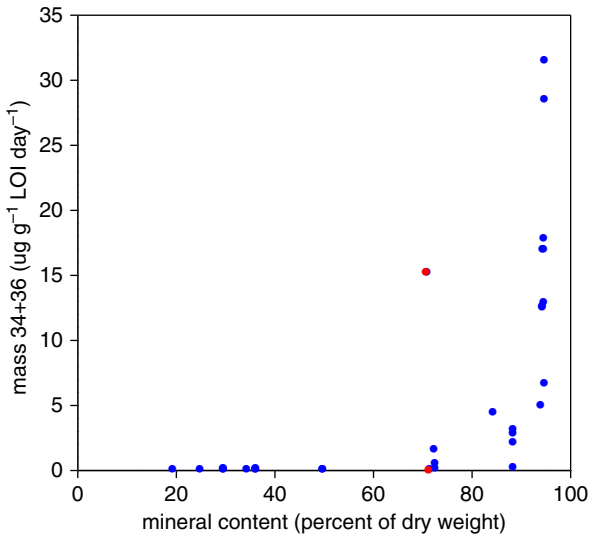

Fig. 1. Sum of masses 34 and 36 produced in soil organic material (LOI) versus soil inorganic material. The deviating value (upper red dot) is from the forest research site Skällås 2009 (two years after P–K fertilisation). Another two years later mass 34 + 36 was not detected at this site (lower red dot).

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