

Dark oxidation of water in soils

By SIEGFRIED FLEISCHER^{1*}, LOVISA BAUHN², PATRIK FORS³ and
ARVID ÖDEGAARD-JENSEN², ¹Rydberg Laboratory, Halmstad University,
P.O. Box 823, SE-301 18 Halmstad, Sweden; ²Department of Chemical and Biological
Engineering/Nuclear Chemistry, Chalmers University of Technology, SE-412 96 Gothenburg,
Sweden; ³Vattenfall Research and Development AB, SE-401 27 Gothenburg, Sweden

(Manuscript received 23 January 2013; in final form 19 November 2013)

ABSTRACT

We report the release of oxygen (O₂) under dark conditions in aerobic soils. This unexpected process is hidden by respiration which constitutes the dominating reversal O₂ flux. By using H₂¹⁸O in different soils, we confirmed that ¹⁶O¹⁸O and ¹⁸O₂ released under dark soil conditions originated from added H₂¹⁸O. Water is the only large-scale source of electrons for reduction of CO₂ 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 O₂.

Keywords: dark water oxidation, soil CO₂ 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 (O₂) 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 O₂ production has been discovered. This is understandable since the O₂-evolving complex is one of the strongest biological oxidation systems known (Wydrzynski, 2008). Under anaerobic conditions, O₂ 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 O₂ as an intermediate (Ettwig et al., 2010, 2012).

1.2. Initial observations

Previous studies indicated that a part of CO₂ in the soil atmosphere was allocated into an internal soil CO₂ 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 CO₂. The mechanism of this process remains speculative; however, the supply of NH₄⁺ stimulated the use of CO₂ as a carbon source thus indicating that nitrification was the possible chemoautotrophic process involved. Therefore, consumption of O₂ was expected to increase as a result of the O₂-consuming NH₄⁺ additions, but instead it decreased. This might be interpreted as within-soil release of O₂. 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 CO₂, 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 CO₂ using H₂O as an electron donor does not coincide with our current knowledge. This implies that the release of O₂ is masked by the predominating O₂ consumption in soils.

*Corresponding author.
email: siegfried.fleischer@hh.se

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 $^{18}\text{O}_2$?

2. Materials and methods

We used sieved (2 mm) composite soil samples ($\phi 25$ 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. $\pm 2^\circ\text{C}$ 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 $^{18}\text{O}_2$ and $^{16}\text{O}^{18}\text{O}$ 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 $\text{BG} + 3 \cdot \text{SD}_{\text{BG}}$ was used as a limit of qualitative determination, and the average $\text{BG} + 10 \cdot \text{SD}_{\text{BG}}$ 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 $^{18}\text{O}_2$ 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 $^{18}\text{O}_2$ and $^{18}\text{O}^{16}\text{O}$ 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 $^{18}\text{O}_2$ or $^{18}\text{O}^{16}\text{O}$ occurred. Release of $^{18}\text{O}_2$ and $^{18}\text{O}^{16}\text{O}$ 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 $^{18}\text{O}_2$ or $^{18}\text{O}^{16}\text{O}$ 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 ₂ O	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
Grillenbug 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 ³⁶S, but this would require that elemental sulphur in the gas phase, which is highly unlikely. A small fraction of ³⁶Ar 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 ¹⁸O₂ and ¹⁸O¹⁶O occurred in soils with pH > 5. ³⁵Cl and ³⁷Cl 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 ¹⁸O₂ 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 ¹⁸O₂ present in inhibited samples. Since this is not the case, ¹⁸O₂ was only detected in biologically active samples, the most probable explanation is that a biological process is the source of ¹⁸O₂.

Some biological processes, which can result in O₂ 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, H₂¹⁸O₂ is formed from the reduction of ¹⁸O₂ which, in turn, must first be released by the indicated oxidation of H₂¹⁸O.

Table 2. Release of $^{18}\text{O}_2$ and $^{18}\text{O}^{16}\text{O}$ after dark incubation of H_2^{18}O in different soils during autumn 2009

Site	Sampling date 2009	Incubation (days)	$^{18}\text{O}_2$ [$\mu\text{g}/(\text{g}_{\text{LOI}}\cdot\text{d})$]	$^{18}\text{O}^{16}\text{O}$ [$\mu\text{g}/(\text{g}_{\text{LOI}}\cdot\text{d})$]
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
Grillenbourg	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 $^{18}\text{O}_2$ 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 $^{18}\text{O}_2$ and $^{18}\text{O}^{16}\text{O}$, and simultaneous soil respiration estimated as decrease in $^{16}\text{O}_2$ after dark incubation of H_2^{18}O in different soils during the summer 2011

Site	Sampling date	Incubation (days)	$^{16}\text{O}_2$ cons. [$\mu\text{g}/(\text{g}_{\text{LOI}}\cdot\text{d})$]	$^{18}\text{O}_2$ prod. [$\mu\text{g}/(\text{g}_{\text{LOI}}\cdot\text{d})$]	$^{16}, ^{18}\text{O}_2$ prod. [$\mu\text{g}/(\text{g}_{\text{LOI}}\cdot\text{d})$]
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
Grillenbourg	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 N₂ and O₂ instead of reduction to N₂O. The O₂ 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 O₂ 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 O₂ in the soil. After 41 d of H₂¹⁸O-incubation in darkness the relation of ¹⁸O¹⁶O to ¹⁸O₂ was still high in some of the samples, that is, the share of H₂¹⁶O 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 O₂ 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.

5. Acknowledgements

The authors thank Karsten Pedersen for advice early in the project, L.-G. Franzén for regular discussions on possible metabolic pathways, Gunnar Jacks for assistance with soil texture descriptions, Christian Ekberg for his comments and advice and four unknown referees for valuable comments. This work was supported by the Brita and Sven Rahmn Foundation.

References

- Currie, L. A. 1968. Limits for qualitative detection and quantitative determination. Application to radiochemistry. *Anal. Chem.* **40**, 586–593.
- Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Manganot, S. and co-authors. 2010. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature*. **464**, 543–548.
- Ettwig, K. F., Speth, D. R., Reimann, J., Wu, M. L., Jetten, M. S. M. and co-authors. 2012. Bacterial oxygen production in the dark. *Front. Microbiol.* **3**, 1–8.
- Fleischer, S. 2012. Interaction between N and C in soil has consequences for global carbon cycling. *J. Resour. Ecol.* **3**, 16–19.
- Fleischer, S. and Bouse, I. 2008. Nitrogen cycling drives a strong within-soil CO₂-sink. *Tellus B.* **60**, 782–786.
- Gerstberger, P. (ed.) 2001. Die BITÖK-Untersuchungsflächen in Fichtelgebirge und Steigerwald. *Bayreuter Forum Ökologie*, 90, 194 pp. ISSN 0944-4122.
- Hagopian, D. S. and Riley, J. G. 1998. A closer look at the bacteriology of nitrification. *Aquacultural Eng.* **18**, 223–244.
- Kalbitz, K., Schwesig, D., Rethemeyer, J. and Matzner, E. 2005. Stabilization of dissolved organic matter by sorption to the mineral soil. *Soil. Biol. Biochem.* **37**, 1319–1331.
- Klaus, S. and Stephan, T. 1998. *Nationalpark Hainich. Laubwaldpracht im Herzen Deutschlands*. RhinoVerlag, Arnstadt & Weimar, 160 pp. ISBN 3-932081-05-6.
- Knohl, A., Schultze, E.-D., Kolle, O. and Buchmann, N. 2003. Large carbon uptake by an unmanaged 250-year-old deciduous forest in central Germany. *Agric. Forest Meteorol.* **118**, 151–167.
- Liu, J., Aronsson, H., Blombäck, K., Persson, K. and Bergström, L. 2012. Long-term measurements and model simulations of phosphorus leaching from a manured sandy soil. *J. Soil. Water Conservat.* **67**, 101–110.
- Martens-Habbena, W., Berube, P. M., Urakawa, H., de la Torre, J. R. and Stahl, D. A. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. *Nature*. **461**, 976–979.

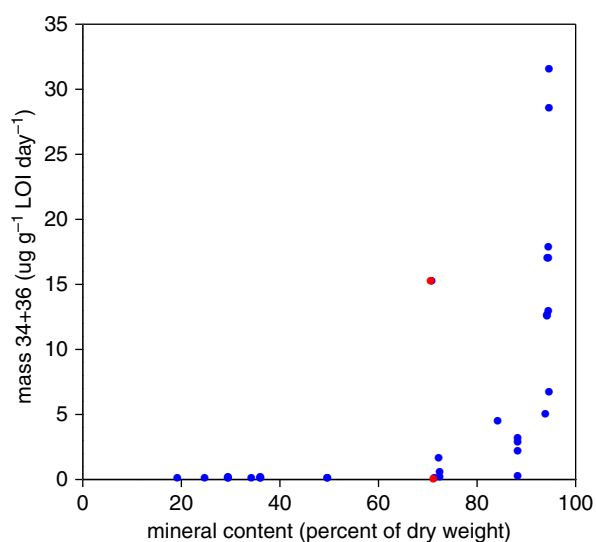


Fig. 1. Sum of masses 34 and 36 produced in soil organic material (LOI) versus soil inorganic material. The deviating value (upper red dot) is from the forest research site Skällås 2009 (two years after P–K fertilisation). Another two years later mass 34 + 36 was not detected at this site (lower red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

- Nilsson, T., Rova, M. and Smedja-Bäcklund, A. 2013. Microbial metabolism of oxochlorates: a bioenergetics perspective. *Biochim. Biophys. Acta*. **1827**, 189–197.
- Pamatmat, M. M. 1997. Non-photosynthetic oxygen production and non-respiratory oxygen uptake in the dark: a theory of oxygen dynamics in plankton communities. *Mar. Biol.* **129**, 735–746.
- Schwärzel, K., Menzer, A., Clausnitzer, F., Spank, U., Häntzschel, J. and co-authors. 2009. Soil water content measurements deliver reliable estimates of water fluxes: a comparative study in a beech and a spruce stand in the Tharandt forest (Saxony, Germany). *Agric. Forest Meteorol.* **149**, 1994–2006.
- Sikström, U. 2005. Pre-harvest soil acidification, liming or N fertilization did not significantly affect the survival and growth of young Norway spruce. *Silva Fennica*. **39**, 341–349.
- Trevors, J. T. 1996. Sterilization and inhibition of microbial activity in soil. *J. Microbiol. Meth.* **26**, 53–59.
- Walker, D. A. 2002. ‘And whose bright presence’ – an appreciation of Robert Hill and his reaction. *Photosynth. Res.* **73**, 51–54.
- Wilson, C. L., Hinman, N. W. and Sheridan, R. P. 2000. Hydrogen peroxide formation and decay in iron-rich geothermal waters: the relative roles of abiotic and biotic mechanisms. *Photochem. Photobiol.* **71**, 691–699.
- Wydrzynski, T. J. 2008. Water splitting by Photosystem II – where do we go from here? *Photosynth. Res.* **98**, 43–51.