**-Supporting Information-**

**HUMIC SUBSTANCES MEDIATE ANAEROBIC METHANE OXIDATION LINKED TO NITROUS OXIDE REDUCTION IN WETLAND SEDIMENTS**

**Running Title:** AOM linked to N2O reduction

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**Table S1.** Experimental conditions implemented in incubations to demonstrate N2O reduction with reduced *Pahokee Peat* Humic Substances (PPHSred) as electron donor.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment name** | **Symbol** | **Reduced PPHS, 1000 mg L-1)** | **Oxidized PPHS, 1000 mg L-1)** | **N2O (4 mL)** | **Autoclave (3 cycles) + chloroform (10%, v/v)** |
| PPHSred/N2O | **♦** | **Marca de verificación** |  | **Marca de verificación** |  |
| PPHSox/N2O | **■** |  | **Marca de verificación** | **Marca de verificación** |  |
| PPHSred (*endogenous*)¶ | **▲** | **Marca de verificación** |  |  |  |
| PPHSred/*Killed*\*  | **x** | **Marca de verificación** |  | **Marca de verificación** | **Marca de verificación** |
| N2O | **●** |  |  | **Marca de verificación** |  |

¶*endogenous* refers to those controls without the addition of N2O acting as electron acceptor, therefore these microcosms’ atmosphere was only composed of argon (99.9% purity, Praxair). \**Killed* controls headspace was spiked with the same volumes of N2O added in the microbially active treatments.

**Table S2.** Description of the experimental conditions and controls implemented in incubations demonstrating CH4 and N2O simultaneous consumption mediated by *Pahokee Peat* Humic Substances (PPHS).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment name** | **Symbol** | ***Pahokee Peat* Humic Substances, 500 mg L-1)** | **N2O (4 mL)** | **13CH4 (2 mL)** | **Autoclave (3 cycles) + chloroform (10%, v/v)** |
| PPHS (*endogenous*)¶ | **▲** | **Marca de verificación** |  |  |  |
| PPHS/13CH4/N2O | **■** | **Marca de verificación** | **Marca de verificación** | **Marca de verificación** |  |
| PPHS/N2O | **♦** | **Marca de verificación** | **Marca de verificación** |  |  |
| PPHS/13CH4 | **●** | **Marca de verificación** |  | **Marca de verificación** |  |
| PPHS/*Killed*\*  | **x** | **Marca de verificación** | **Marca de verificación** | **Marca de verificación** | **Marca de verificación** |
| Sediment (*endogenous*)¶ | **▲** |  |  |  |  |
| 13CH4/N2O | **■** |  | **Marca de verificación** | **Marca de verificación** |  |
| N2O | **♦** |  | **Marca de verificación** |  |  |
| 13CH4 | **●** |  |  | **Marca de verificación** |  |
| *Killed*\*  | **x** |  | **Marca de verificación** | **Marca de verificación** | **Marca de verificación** |

¶*endogenous* refers to microcosms without the addition of gases acting as electron donor or acceptor (13CH4 or N2O), therefore these microcosms’ atmosphere was only composed of argon (99.9% purity, Praxair). \**Killed* controls headspace was spiked with the same volumes of 13CH4 and N2O added in the microbially active treatments.



**Figure S1. GC-MS chromatograms showing qualitative evidence of the reduction of 15N2O to 30N2 (15,15N2) with PPHSred as electron donor.** Superior panels display signals extracted for the ion mass equivalent to 30 in which the signal for 30N2 can be found at the retention time of ~1.8 min. Inferior panels display the chromatograms extracted for the ion mass equivalent to 46 in which the signal for 30N2O can be found at the retention time of ~2.3 min.



**Figure S2. Nitrous oxide reduction promoted by *Pahokee Peat* Humic Substances (PPHS) acting as electron shuttle and 13CH4 as electron donor during the first cycle of incubation. Panel A** depicts the normalized (concentration / initial concentration, C/Ci) kinetics of N2O consumption with and without PPHS as electron shuttle. **Panel B** shows the maximum N2O reduction rates (bars, left axis) based on the linear regressions of at least three sampling points during the period of highest activity. The net amount of N2O depleted after 8 days of incubation is shown in the right axis (**•** symbols). Data represent the average from triplicate incubations ± standard error. \*Killed controls contain the same concentration of 13CH4 and N2O as in the main experimental treatments. Statistically different treatments are represented with letters obtained via a one-way ANOVA and the Duncan post hoctest (95% percent confidence interval).



**Figure S3. Standard Gibbs free energy (ΔG°`, considering pH = 7) of the humic substances (HS)-dependent or -mediated reactions examined in this work.** Calculations were made according to Nernst equation considering the reduction of N2O to N2 (**Eq. 1 and 3**), the oxidation of 13CH4 to 13CO2 (**Eq. 2 and 3**), and the whole range of standard redox potentials (E°`) reportedfor HS (data taken from references)1,2:

**ΔG°` = - n \* F \* ΔE°`**

**Where:**

**n** = number of electrons transferred during the redox reaction per mol of N2O or 13CH4 = 2 or 8, respectively.

**F** = Faraday constant = 96. 56 kJ/V-mol

ΔE°` = Difference in standard redox potential between electron acceptor and electron donor

**For Equation 1:**

ΔE°`= (E°`N2O/N2) – (E°`HSox/HSred)

E°` for Humic Substances = ¶from -0.3 V to +0.3 V

E°` for couple N2O/N2 = +1355 V

**For Equation 2:**

ΔE°`= (E°`HSox/HSred) – (E°`CH4/CO2)

E°` for Humic Substances = ¶from -0.3 V to +0.3 V

E°` for couple CH4/CO2 = -240 V

**For Equation 3:**

ΔE°`= (E°`N2O/N2) – (E°`CH4/CO2)

E°` for couple N2O/N2 = +1355 V

E°` for couple CH4/CO2 = -240 V

**References**:

(1) Straub, K. L.; Benz, M.; Schink, B. Iron metabolism in anoxic environments at near neutral pH. *FEMS Microbiol. Ecol.* **2000**, *34* (3), 181–186.

(2) Aeschbacher, M.; Vergari, D.; Schwarzenbach, R. P.; Sander, M. Electrochemical analysis of proton and electron transfer equilibria of the reducible moieties in humic acids. *Environ. Sci. Technol.* **2011**, *45* (19), 8385–8394.