**Supplementary Information**

# Mitigating fungi-related nitrous oxide emissions by application of industrial waste in an acidic soil

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**Soil properties analysis**

In a parallel experiment, a total of 60 soil samples with 4 treatments (CK, U, A, A+U) of three repeats were incubated in 120 ml serum flasks for soil pH, mineral N, and dissolved organic carbon (DOC) and nitrogen (DON) analysis at 24, 48, 96, 120 and 184 h conducted in closed bottles with 21% initial oxygen (O2) concentration. Soil pH was measured in distilled water with soil/solution ratio of 1:2.5 using a pH meter (MP522 version 3, SANXIN, China). Soil was extracted using 1 M KCl solution at a soil: KCl solution ratio of 1:5 (w/v) and exchangeable ammonium (NH4+-N) and nitrate (NO3--N) concentrations were determined using a continuous flow analyser (AA3, Seal Analytical, Norderstedt, Germany). Soil nitrite (NO2--N) concentrations were determined by a spectrophotometer (UVmini-1240, Shimadzu, Japan) at 543 nm. Soil was extracted using 0.5 M K2SO4 solution for DOC and DON analysis, and the extract was filtered through a 0.45 μm membrane and measured using a TOC/TN analyser (TOC-VCSH, Shimadzu, Kyoto).

Extracellular enzyme activities from the soil were established in opened 0.7-L cylindrical incubation containers (diameter: 80 mm, height: 88 mm) with 100 g of air-dried soils with 3 treatments. The moisture concentration (70% WHC) was replenished by adding deionized water at the beginning of the incubation. The containers were covered with perforated parafilm and placed in an incubator under 25°C in the dark. Soil extracellular enzyme activities was measured after 2 days incubation. Soil extracellular enzyme activities were measured with fluorogenically labeled artificial substrates according to (Turner, 2010) with a multimode reader (Varioskan flash, Thermo Scientific, USA). Fluorogenic 4-methylumbelliferone (MUB)-based substrate was used to determine the activities of β-1, 4-glucosidase (EC 3.2.1.21), β-cellobiosidase (3.2.1.91), N-acetyl-β-glucosaminidase (EC 3.2.1.30). Fluorogenic 7-amino-4-methylcoumarin (AMC)-based substrate was used to determine the activity of L-leucine aminopeptidase (EC 3.4.11.1).

**Optimum inhibitor concentrations selection**

Five concentrations of 1, 4.5, 6, 10, and 12 mg g-1 dry soil were used into cycloheximide and streptomycin respectively prior to the experiment to assess the optimal concentration of the inhibitors and avoid the inhibitors affecting non-target microorganisms. All flasks were immediately sealed with air-tight bromobutyl rubber septa and aluminum caps (Macherey-Nagel, Germany). CO2 emissions were sampled at 4 h and 8 h after the addition of glucose (Maet al., 2017). The inhibitor additivity ratio (IAR) was used to evaluate the non-target microorganism inhibition by cycloheximide and streptomycin (Beareet al., 1990). The optimum inhibitor concentrations were calculated based on the IAR as Equation (1). The optimum combined concentrations were selected when the ratio approached 1. Each treatment has three replicates.

[(A − B) + (A − C)]/(A − D)] (1)

A is the CO2 production rate in the soil amended with glucose only, B is the CO2 production rate in the soil amended with glucose and streptomycin; C is the CO2 production rate in the soil amended with glucose and cycloheximide; and D is the CO2 production rate in the soil amended with glucose, cycloheximide and streptomycin. The optimum concentrations were 6 mg g−1 soil with corresponding IAR values of 1.03.

**References:**

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Table S1 Soil properties used in this study

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Clay(%) | Silt(%) | Sand(%) | pH | EC(μS cm-1) | OC(g kg-1) | TN(g kg-1) | C/N | Olsen-P(mg kg-1) | Available K (mg kg-1) |
| 11.2 | 58.5 | 30.3 | 4.80 | 94.4 | 26.6 | 2.42 | 11.0 | 44.9 | 80.0 |

Table S2 Chemical composition of the amendment used

|  |  |  |  |
| --- | --- | --- | --- |
| Element | Content % | Element | Content % |
| Ca | 53.67 | Si | 18.4 |
| Al | 7.85 | K | 6.5 |
| Fe | 5.66 | Mg | 1.49 |
| CaO | 41.74 | SiO2 | 27.75 |
| Al2O3 | 10.99 | SO3 | 6.13 |
| K2O | 4.72 | Fe2O3 | 3.9 |
| MgO | 1.87 | P2O5 | 1.46 |



Fig. S1 The cumulative CO2 (A) and N2O (B) emissions and global warming potential (GWP) (C) as affected by the mineral treatments after 88 h incubation. The different lowercases show the significant differences among treatments (*P*<0.05).