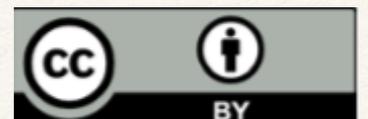


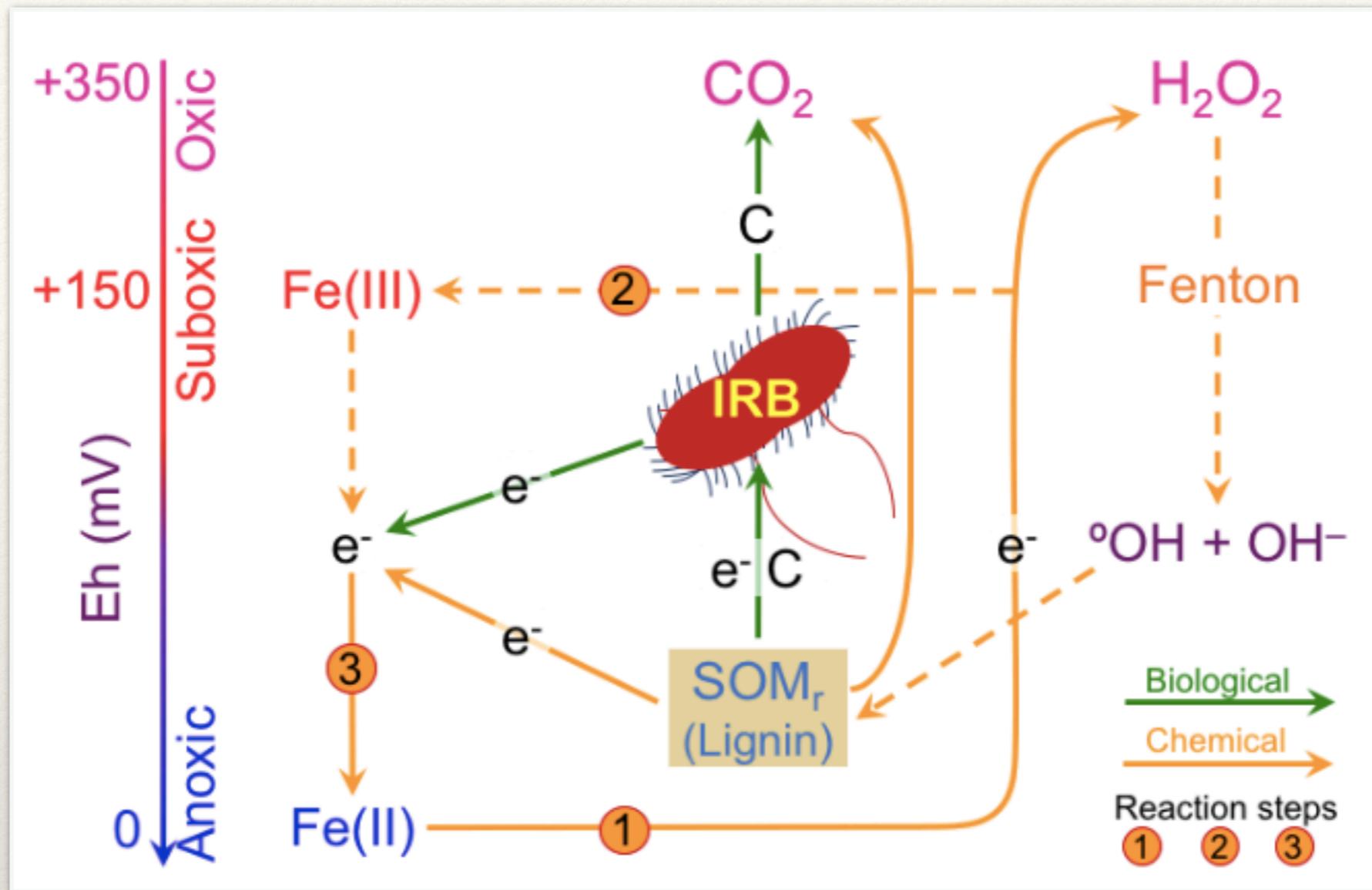
# Iron-reducing bacteria play a key role in lignin degradation by electron transferring from soil organic matter

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Electron acceptors ( $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Fe}^{3+}$ ,  $\text{Mn}^{4+}$ ) play a crucial function in the oxidation of soil recalcitrant organic compounds. Soils that present large amount of total Fe (8-57 g  $\text{kg}^{-1}$  soil) and organic (C) (10-110 g  $\text{kg}^{-1}$  soil), iron-reducing bacteria (IRB) may play a important role. In the present study we hypothesized that IRB which reduce Fe(III)(oxyhydr)oxide of low solubility to soluble Fe(II), can contribute substantially to the degradation of lignin from soil organic matter (SOM). The aim of this study was to isolate IRB and evaluate their importance in lignin degradation. IRB were obtained from topsoils of different climates (humid temperate, cold temperate, subpolar), vegetation type (steppe, rainforest) and parent materials (granitic, volcanic, fluvio-glacial, basaltic-Antartic and metamorphic). The potential of IRB to reduce Fe(III) was assessed with lactate substrate as source of carbon (C) and anthraquinone-2,6-disulfonate (AQDS) as electron acceptor. The contribution of IRB to lignin degradation was assessed in an anaerobic microcosms experiment for 36 h. The  $\text{CO}_2$  efflux from sterilized and reinoculated soil with IRB was compared with sterilized (abiotic), non-sterilized (biotic) and induced Fenton reaction. Lignin degradation by IRB was examined by: 1) bacterial growth containing alkali lignin and alkali lignin disappearance during incubation, 2) Lignin peroxidase and manganese peroxidase activities originated from IRB, 3) cells abundance estimated from ATP synthase from bacteria growing in alkali lignin and 4) lignin degradation monitored by fluorescence disappearance intensity. The major microbial group for Fe(III) reduction, as essayed by PLFA and nested-PCR and sequencing different species were Geobacteriaceae-strains (*G. metallireducens* and *G. lovleyi*) in all studied. The  $\text{CO}_2$  respiration in reinoculated soils was 140% higher than the  $\text{CO}_2$  release by abiotic and Fenton reaction and, 40% lower than biotic treated soil. The Fe(II) extractable in HCl in soil derived from basaltic-Antarctic parent material showed 362 % more Fe(II) solubilisation than that of biotic treatment. Fluorescence intensity decreased during lignin degradation and it was closely correlated with  $\text{CO}_2$  release in the same sample. We conclude that IRB community such as *Geobacter* spp. Uses intensively Fe(III) as an electron acceptor to oxidize lignin compounds, and this process is especially active in Fe rich soils.

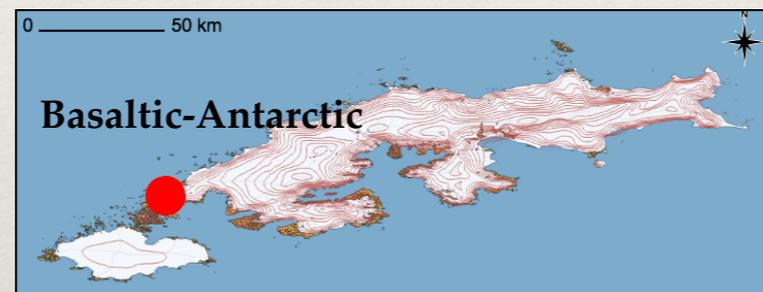
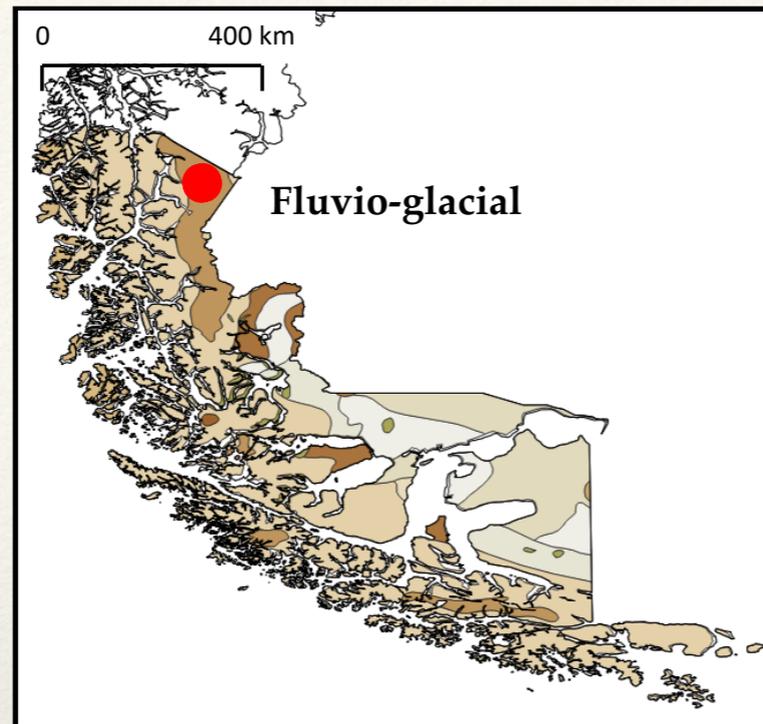
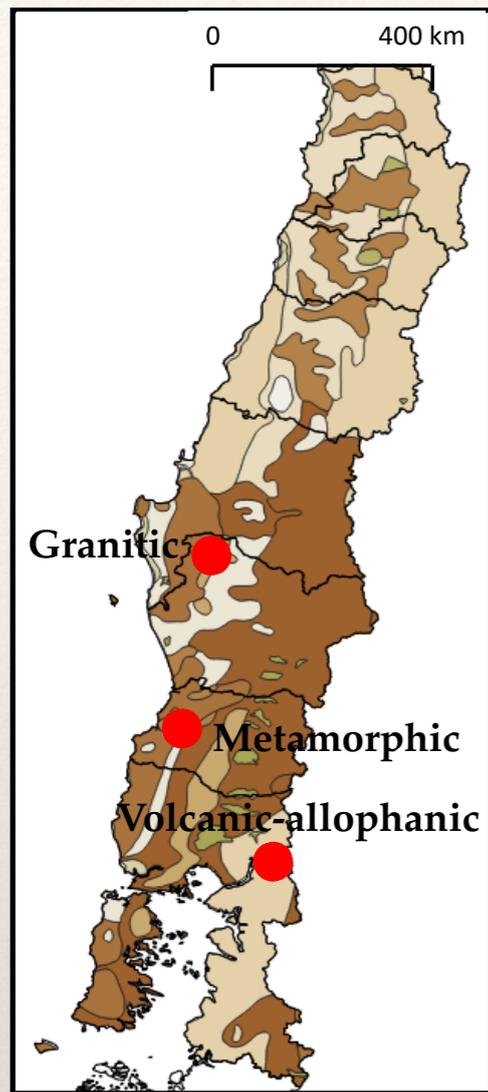


# Graphical Abstract



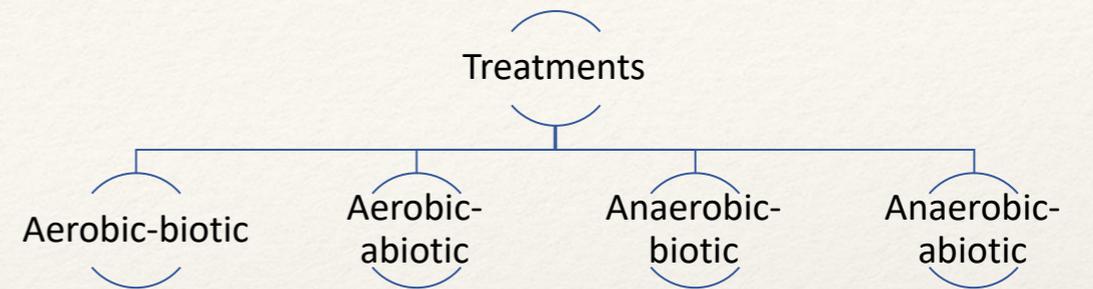
Conceptual model of iron reducing bacteria (IRB) that work from lignin-containing reduced organic matter in the soil (SOM<sub>r</sub>), which in turn serves as an electron donor ( $e^-$ ) and carbon source for IRB cells in a gradient of redox conditions. IRB in turn reduces insoluble Fe(III) (oxyhydr)oxides to soluble Fe(II) yielding CO<sub>2</sub> (Bio). SOM<sub>r</sub> can give an electron directly to Fe(III) (Ch) and Fenton reactions can oxidize hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), produced during aerobic phase, to generate hydroxyl radicals for soil organic C oxidation from SOM. Electron transfer keep ferrous wheel continuing over many redox cycles

# Soils under study on Chilean soil map



## Experimental design

### Experiment 1: (Aerobic-Anaerobic)



### Experiment 2: (Anaerobic-IRB inoculated and Fenton)

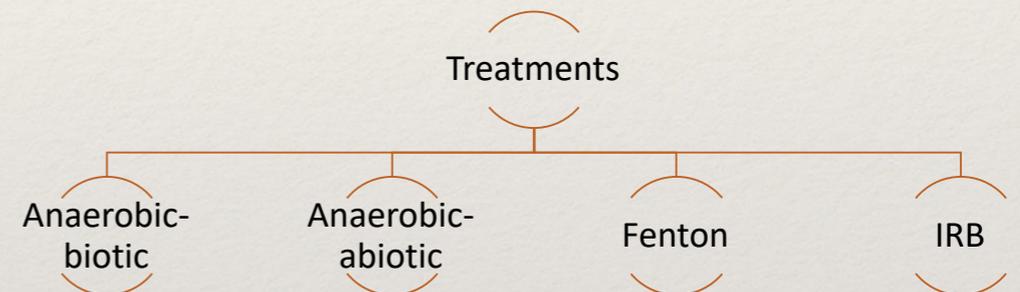
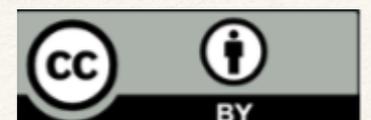
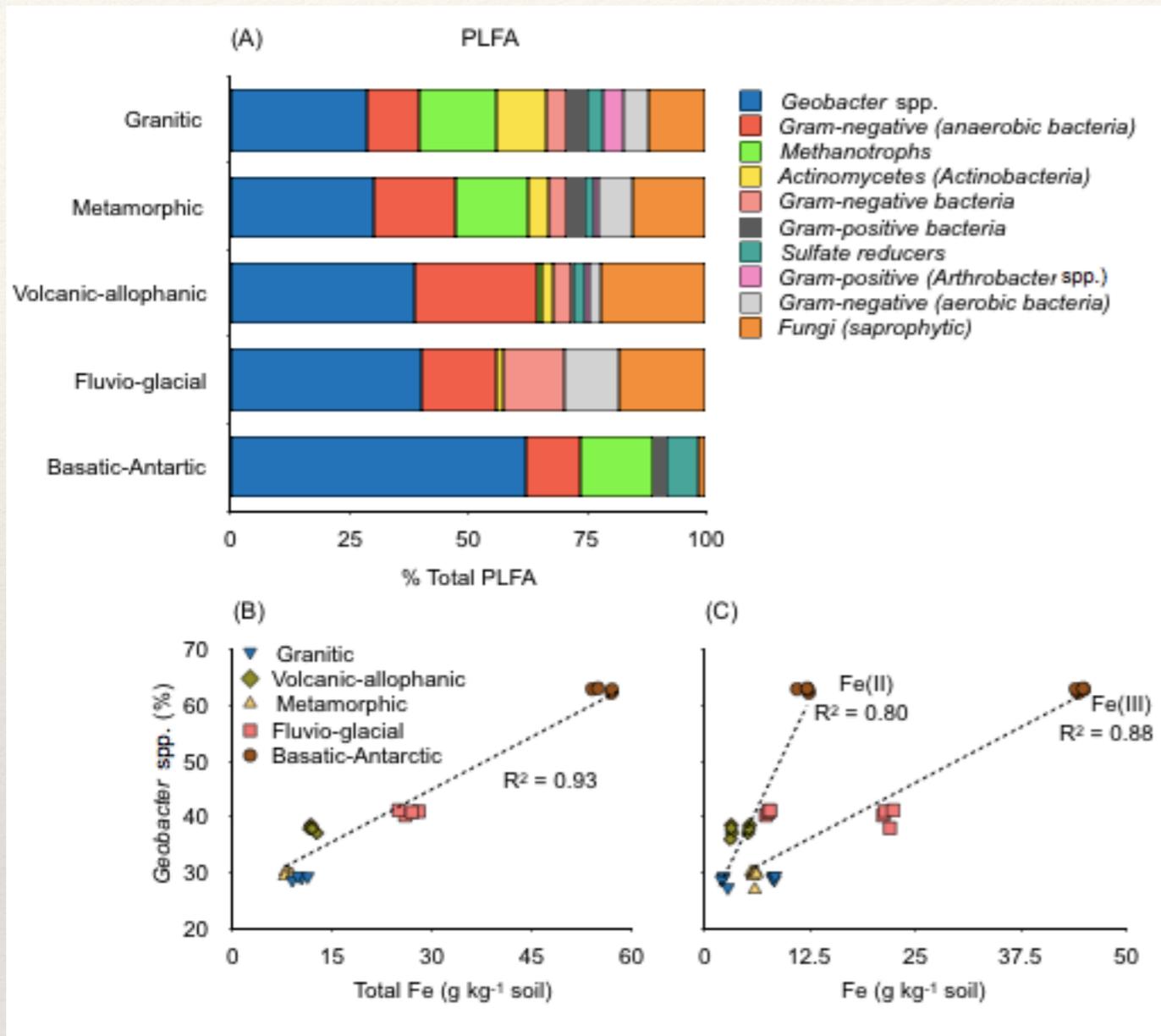


Table 1. Characteristics of the study sites

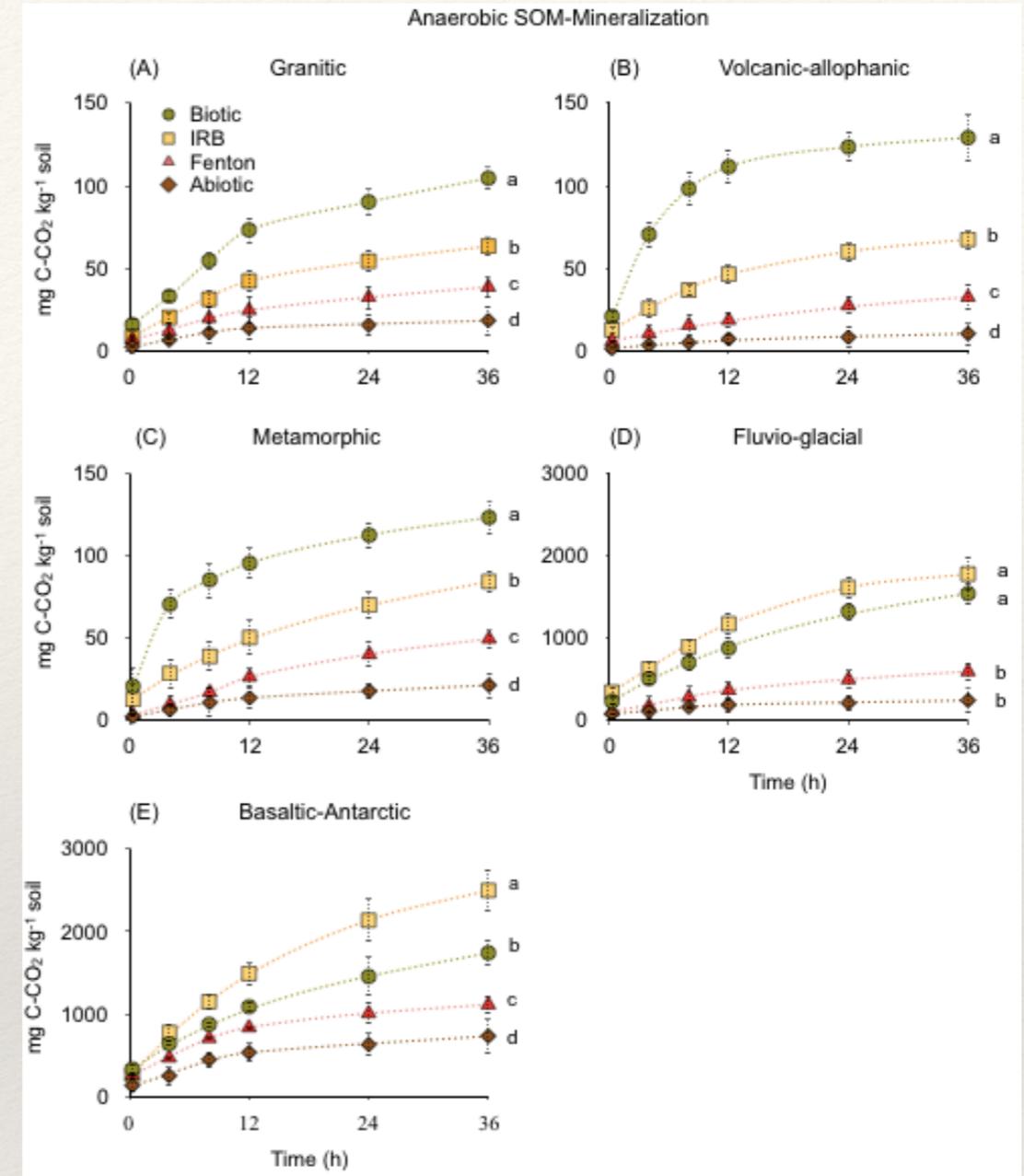
Parent Materials	Coordinates	Elevation (m a.s.l.)	MAT (°C)	MAP (mm)	Climate	Soil order
Granitic	37°47'S / 72°59'W	1000	13.3	1491	Warm-Temperate	Inceptisol
Methamorphic	40°12'S / 73°26'W	1048	9.5	4000	Oceanic	Ultisol
Volcanic-Allophanic	40°47'S / 72°12'W	800	9.2	>5000	Rain temperate	Andisol
Fluvio-glacial	50°58'S / 72°57'W	100	12	684	Subpolar	Inceptisol
Basaltic-Antarctic	62°13'S / 58°59'W	13	-2.2	350	Subpolar	Cryosol



# Results



Correlation between Phospholipid fatty acid (PFLA) and Fe for (B) the total Fe content and (C) Fe reduced (Fe(II)) and Fe oxidized (Fe(III)) species in soils.



CO<sub>2</sub> evolved from soil under anaerobic incubations (12 °C, 36 h) and inoculated with IRB, sterilized only (abiotic), non-sterilized (biotic) and added with a 10:1 of H<sub>2</sub>O<sub>2</sub>:Fe(II) ratio to induce Fenton reaction .

# Conclusions

- ❖ Both, iron abiotic oxidation and IRB contributes to explain the rapid turnover of soil C under anaerobic conditions.
- ❖ Microbial respiration coupled with lignin decomposition under anaerobic conditions was common in all soils and was maximal in soils with high Fe content.
- ❖ Overall climate and vegetation, the respiration of different soil type showed highly dependence on metal reducer microorganism along with lignin degradation in various ecosystems.
- ❖ It is necessary to extend this studies to other type of soils and ecosystems in surface and deep soils.



# Funding

This work was supported by:

- ❖ National Commission of Research of Science and Technology of Chile (CONICYT/ FONDECYT). Fondecyt N° 11180521 and N° 1170119.
- ❖ Network for Extreme Environment Research (NEXER-Chile) N° 2.
- ❖ Government Program of Competitive Growth of Kazan Federal University and by the support of the “RUDN University program 5-100”.