The impacts of biochar and compost on microbial extracellular electron transfer processes as shown by studies on soil microbial fuel cells

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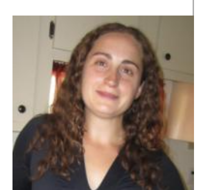

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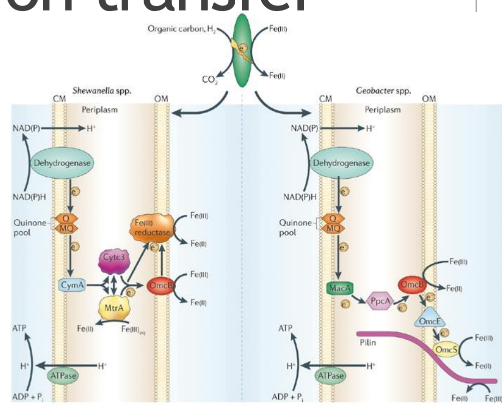
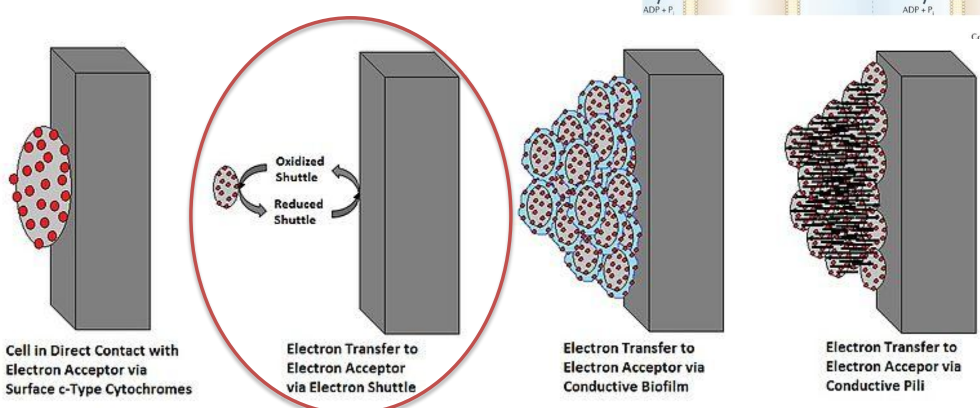
Extracellular electron transfer

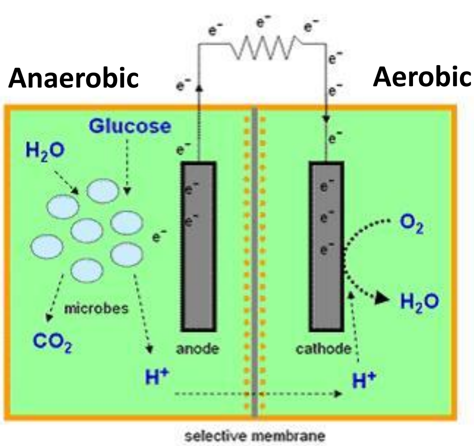
- Extracellular electron transfer (EET): microbial process involved in a specialized form of **anaerobic respiration**
- Anaerobic respiration involving *insoluble or membrane-impermeable* electron acceptors require EET
  - e.g., iron oxides, humic substances, anode electrode in SMFC

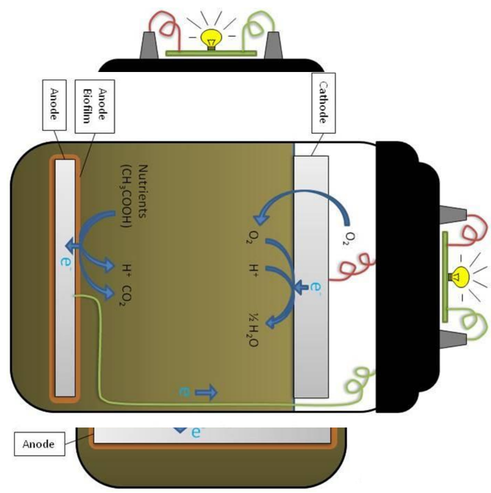


Nature Reviews | Microbiology



Microbial fuel cell: the basics





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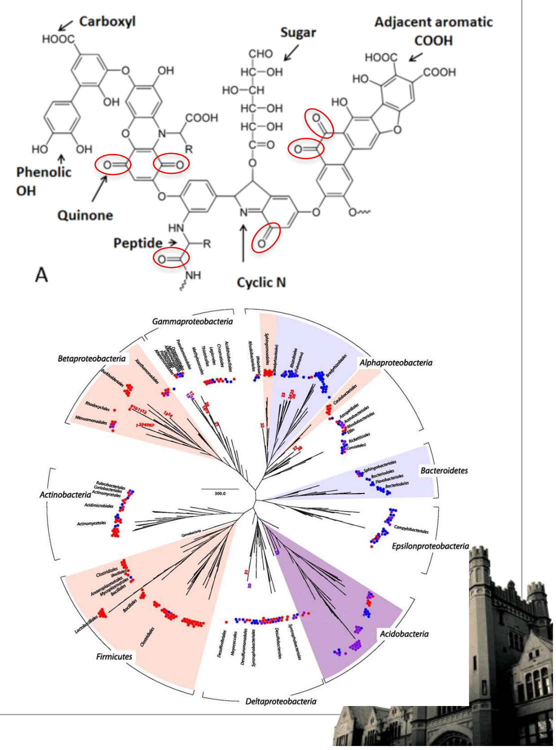
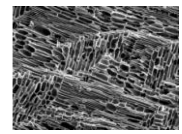
Electron shuttles: humic substances

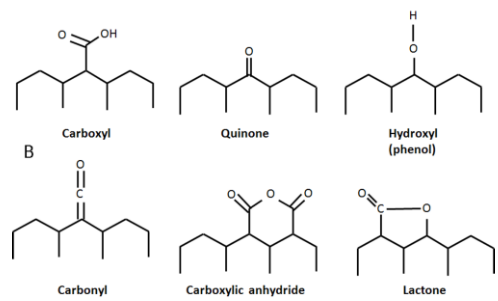
- Recalcitrant and ubiquitous
- Most redox-active group are quinones; hydroquinone = electron donor; quinone = electron acceptor
- Microbes capable of utilizing humic substances as electron donor/acceptor are ubiquitous – example is from agricultural soil (**Van Trump et al. mBio 2011; doi:10.1128/mBio.00044-11**

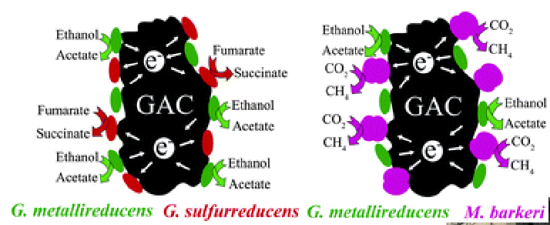


Biochar and EET



- Important similarities to humic substances – quinone groups also present
- Charcoal and similar materials have been used as electrodes for MFCs
- Granular activated carbon can facilitate direct interspecies electron transfer
- Biochar supports EET and biofilm growth





Liu et al. 2012. Energy Environ. Sci

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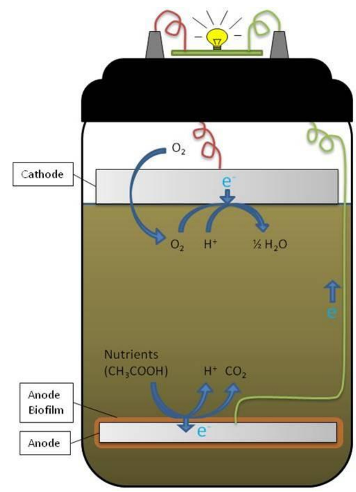
Synergism Hypotheses

- If biochar and HS enriches for microbes involved in EET in biofilm form, then amending soil with biochar and source of HS (compost) will lead to enrichment of anode-reducing bacterial biofilms, which will enhance electricity production than either substance alone
- Similarly, providing anode- and iron-reducing bacteria with biochar and HS will enhance Fe reduction at a higher rate than either alone



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Approach

- Construct SMFCs with the following treatments: soil alone, soil plus biochar, soil plus compost, soil plus biochar plus compost
- Monitor electricity production
- Perform microbial community analysis of anode biofilm using next-generation DNA sequencing
- Use NGS data to select a pure bacterial culture for Fe(III) reduction experiments in defined medium comparing the same treatments

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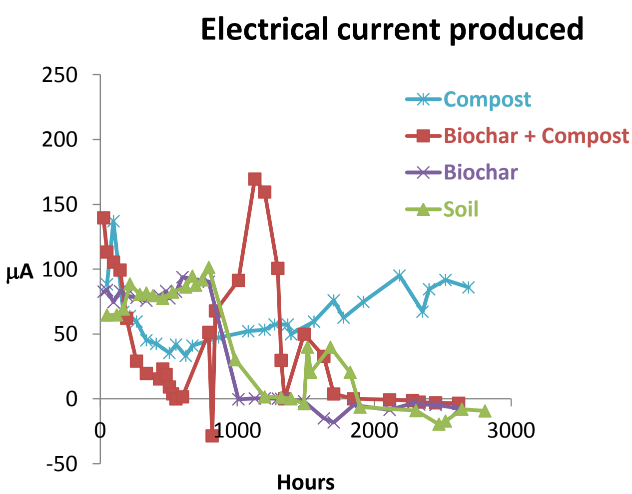
Results: SMFC experiment



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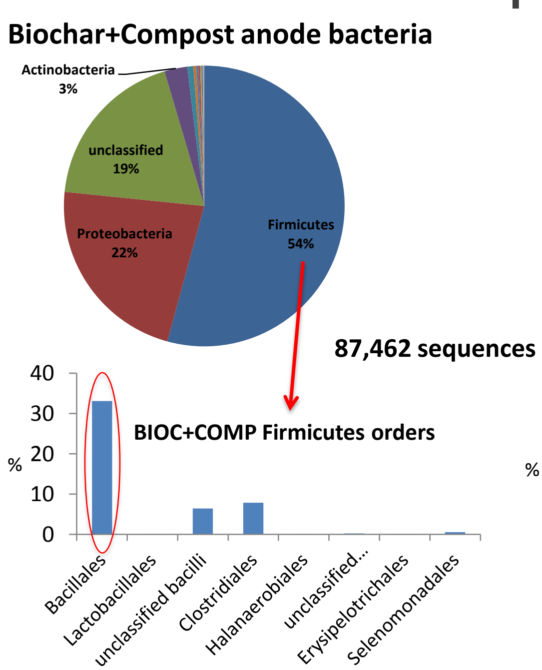
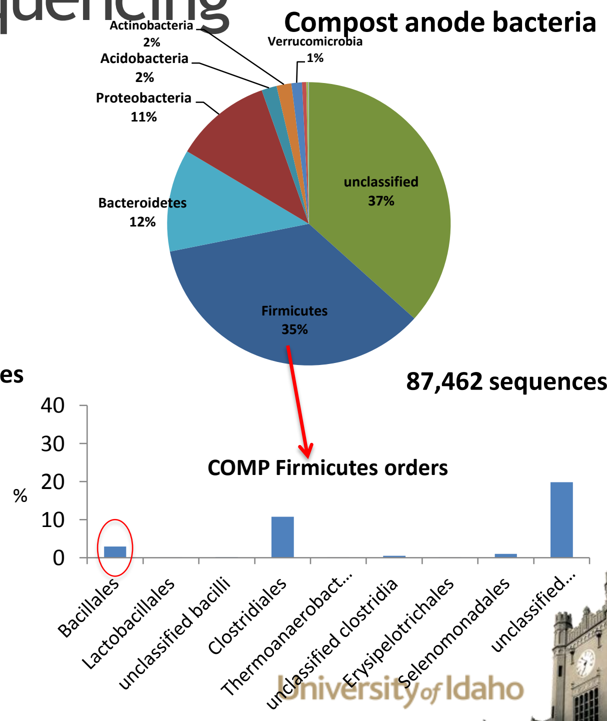
Results: Next-generation DNA sequencing





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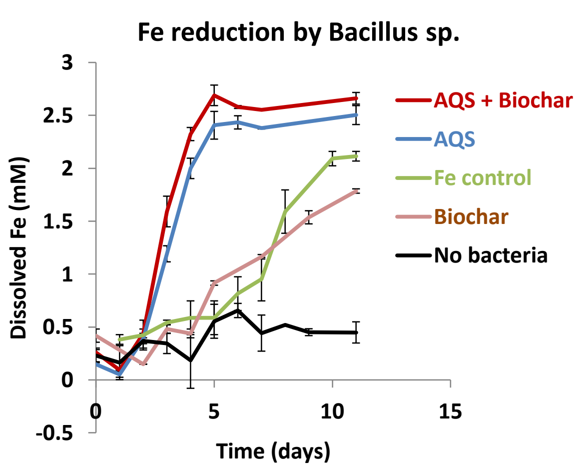
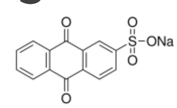
Results: Reduction of Fe(III) by a *Bacillus* sp.

- *Bacillus* sp. – a known Fe(III) reducer; selected based on identification of dominant anode reducer by next-gen DNA sequencing

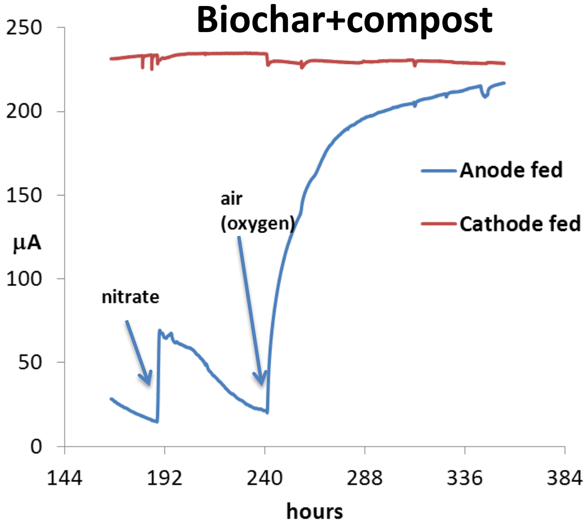




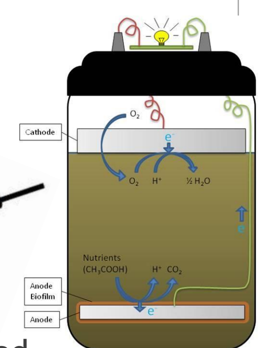
AQS = humic substance analog

Results: Cathode experiments - EET in response to nitrate

Aerobic conditions at cathode
Biochar+compost



- Nitrate injected



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Recap

- Maximum peaks of electricity production are observed in biochar plus compost treatment
- Microbial communities on the anode contain high abundance of *Bacilli* in biochar plus compost vs. compost alone
- Highest rates of Fe(III) reduction by a *Bacillus* sp. observed when biochar and AQS are present at the same time
- Overall supports hypothesis that biochar and HS enhance EET synergistically
- We are now investigating the possibility that biochar and HS also enhance electron transfers involved in nitrate respiration– **lithotrophic denitrification**?

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Acknowledgements

- Funding from University of Idaho Office of Research and Economic Development and USDA Hatch funds
- Haley Egan
- Christopher Currie
- Prof. Dan Strawn

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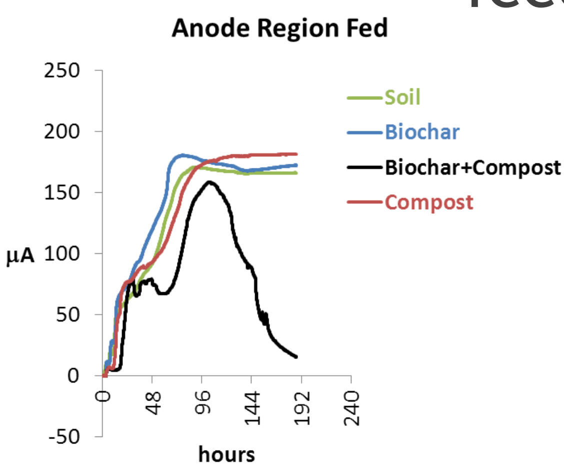
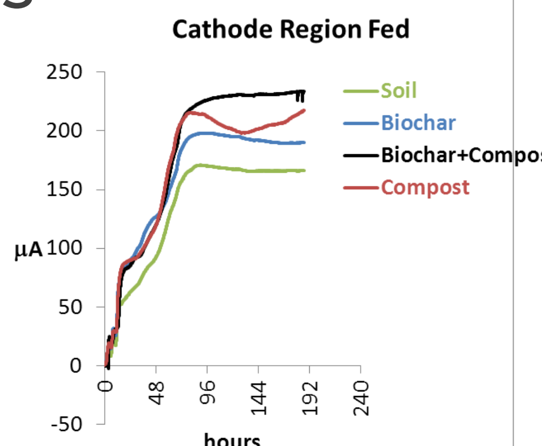
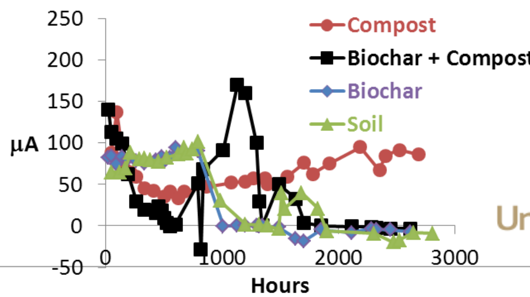
Results: SMFC electrode-specific feeding







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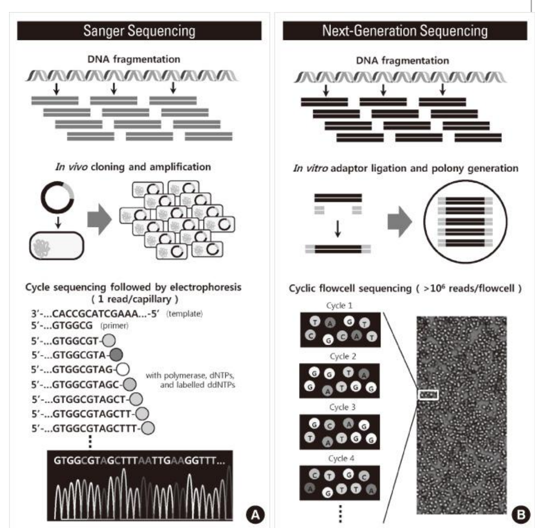
Next-generation DNA sequencing

- “Old” = Sanger sequencing
- NGS = massively parallel approach to DNA sequencing
- A number of different platforms or methods, we are using Ion Torrent



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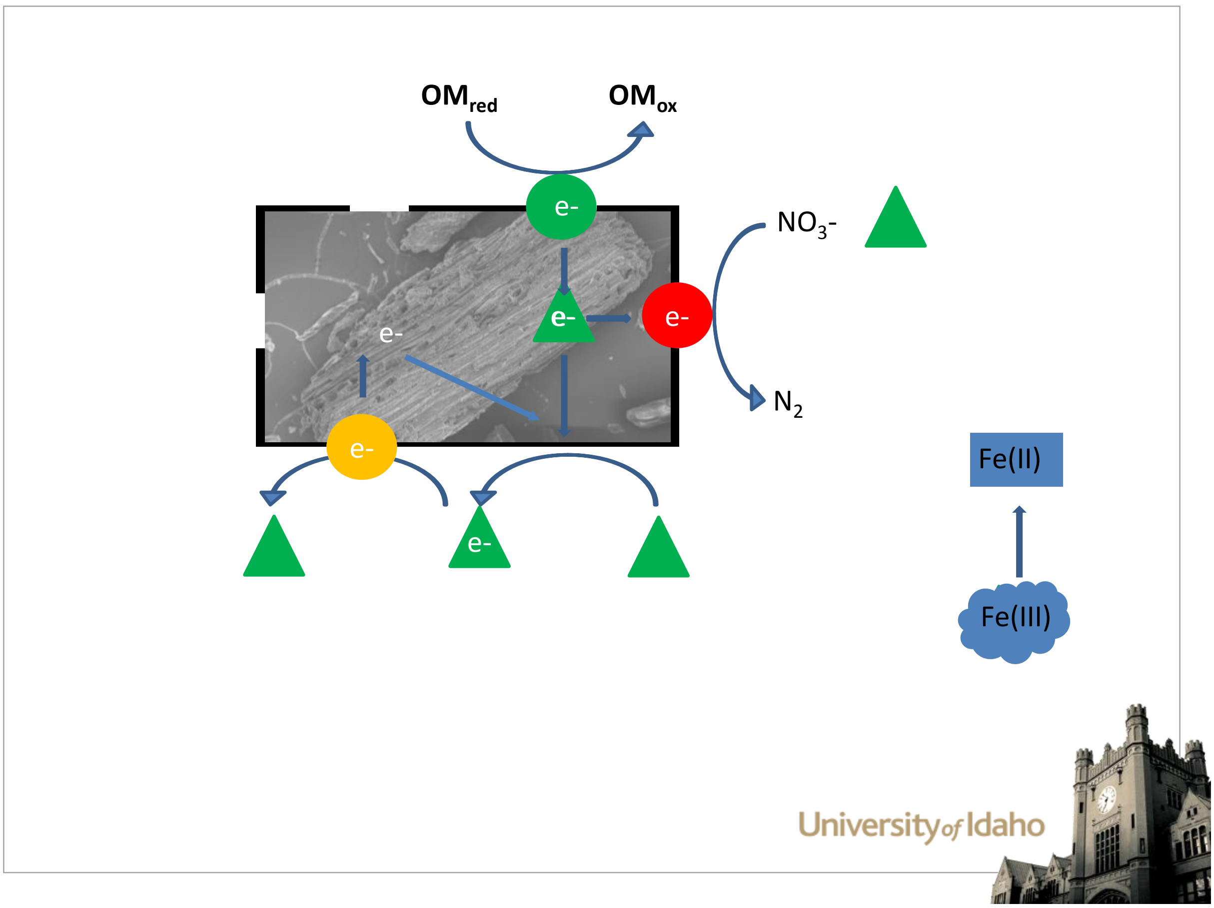

OMred
OMox
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e-
e-
e-
NO3-
N2
e-
e-
Fe(II)
Fe(III)
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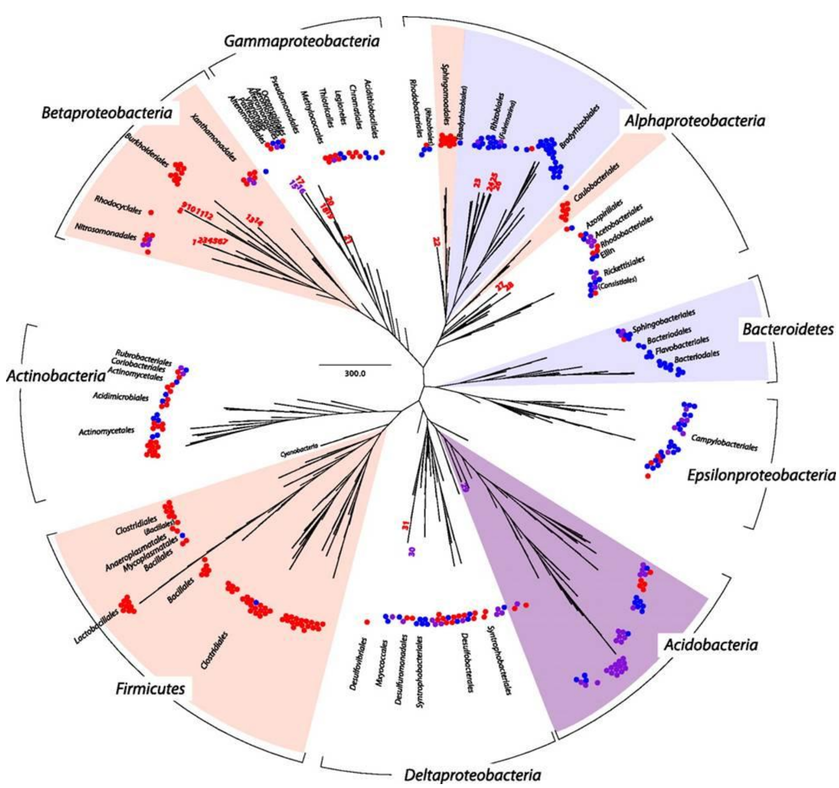

Red = reduced HA

Blue = oxidized HA

Van Trump J I et al. mBio 2011; doi:10.1128/mBio.00044-11

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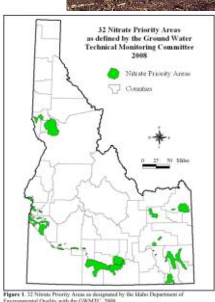
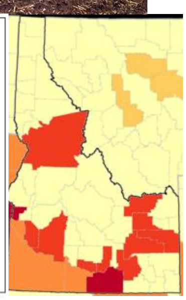
Links between iron, nitrate (N_2O)



Nitrate priority areas Idaho DEQ



Cattle growing areas http://www.factoryfarmmap.org

Iron: The Forgotten Driver of Nitrous Oxide Production in Agricultural Soil

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Abstract

In response to rising interest over the years, many experiments and several models have been devised to understand emission of nitrous oxide (N_2O) from agricultural soils. Notably absent from almost all of this discussion is iron, even though its role in both chemical and biochemical reactions that generate N_2O was recognized well before research on N_2O emission began to accelerate. We revisited iron by exploring its importance alongside other soil properties commonly believed to control N_2O production in agricultural systems. A set of soils from California's main agricultural regions was used to observe N_2O emission under conditions representative of typical field scenarios. Results of multivariate analysis showed that in five of the twelve different conditions studied, iron ranked higher than any other intrinsic soil property in explaining observed emissions across soils. Upcoming studies stand to gain valuable information by considering iron among the drivers of N_2O emission, expanding the current framework to include coupling between biotic and abiotic reactions.

Citation: Zhu X, Silva LCR, Doane TA, Horwath WR (2013) Iron: The Forgotten Driver of Nitrous Oxide Production in Agricultural Soil. PLoS ONE 8(3): e60146. doi:10.1371/journal.pone.0060146

Can biochar play a role in reducing impacts of nitrogen pollution?

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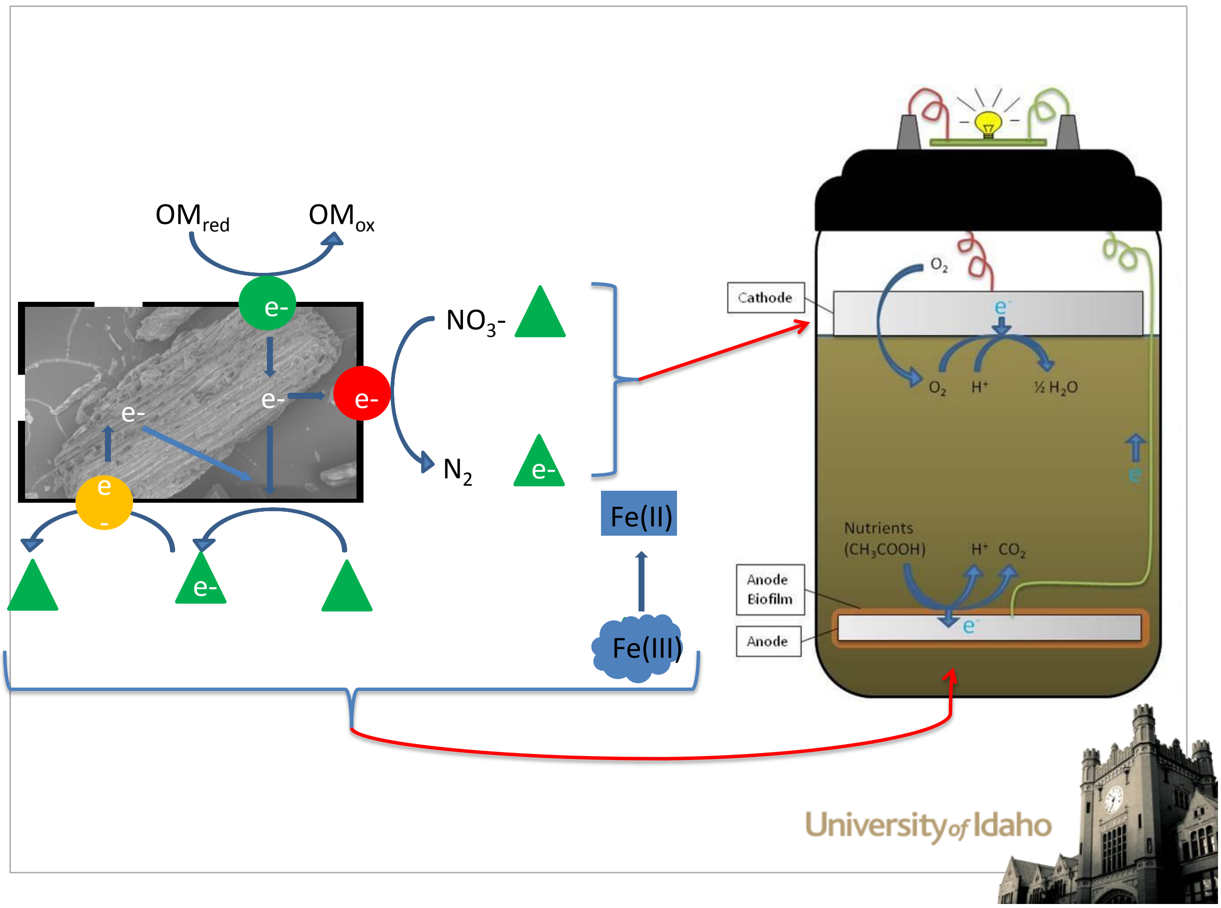

OMred
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NO3-
N2
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Fe(II)
Fe(III)
Cathode
O2
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O2
H+
½ H2O
e-
Nutrients
(CH3COOH)
H+ CO2
Anode Biofilm
Anode
e-
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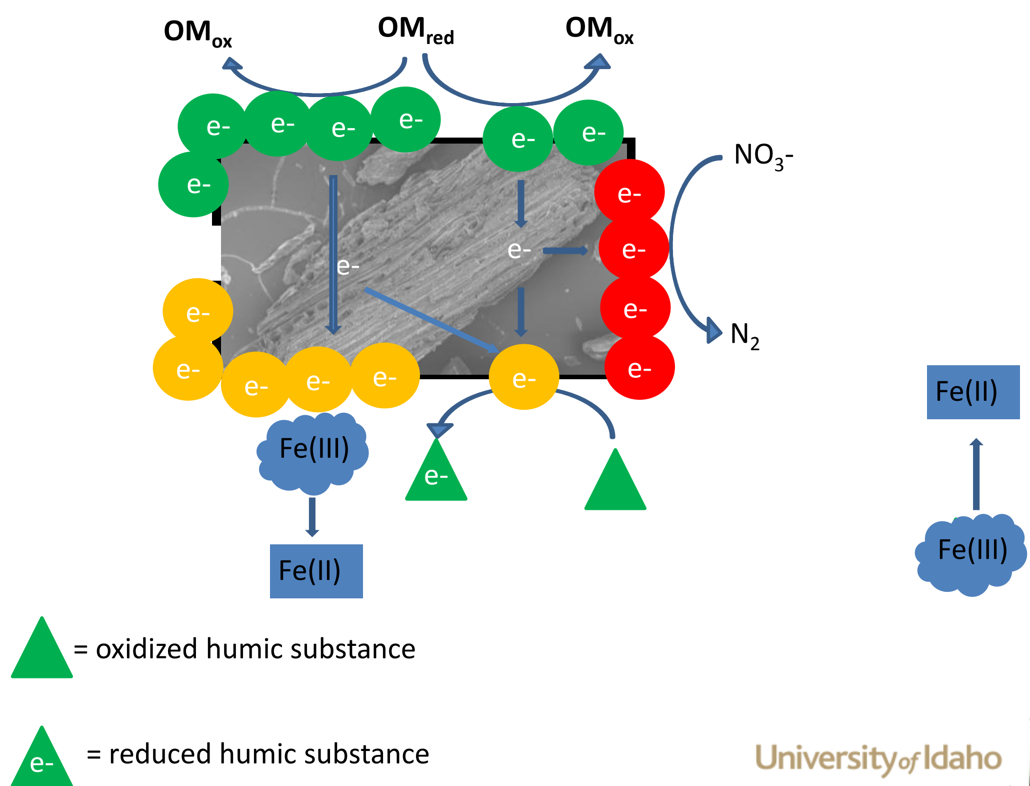

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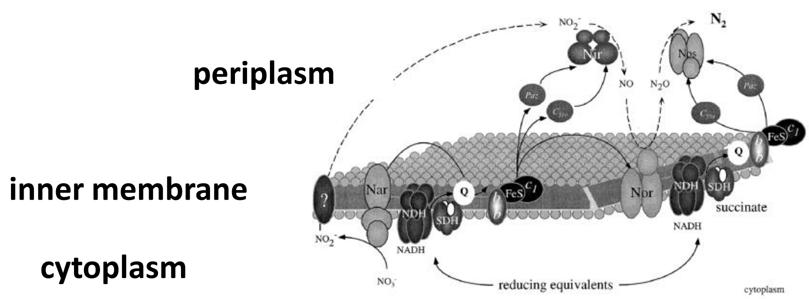
*Intra*cellular electron transfer

- Anaerobic respiration in *P. denitrificans* occurs *intracellularly*
- Involves electron flow driven by strong thermodynamics to synthesize ATP



Baker, S.C., Ferguson, S.J., Ludwig, B., Page, M.D., Richter, O.-M.H., van Spanning, R.J.M.: Molecular genetics of the genus Paracoccus: Metabolically versatile bacteria with bioenergetic flexibility Microbiol. Mol. Biol. Rev. 1998, 62, 1046-1078.

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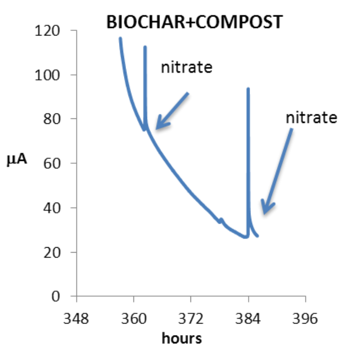
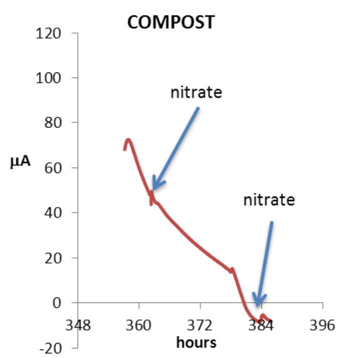
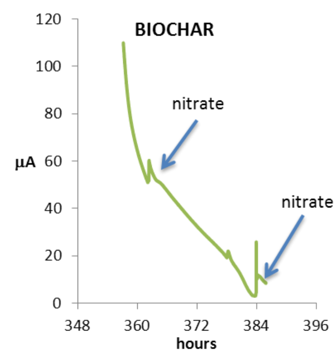
Cathode experiments: Electrical responses to nitrate

Anaerobic conditions







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