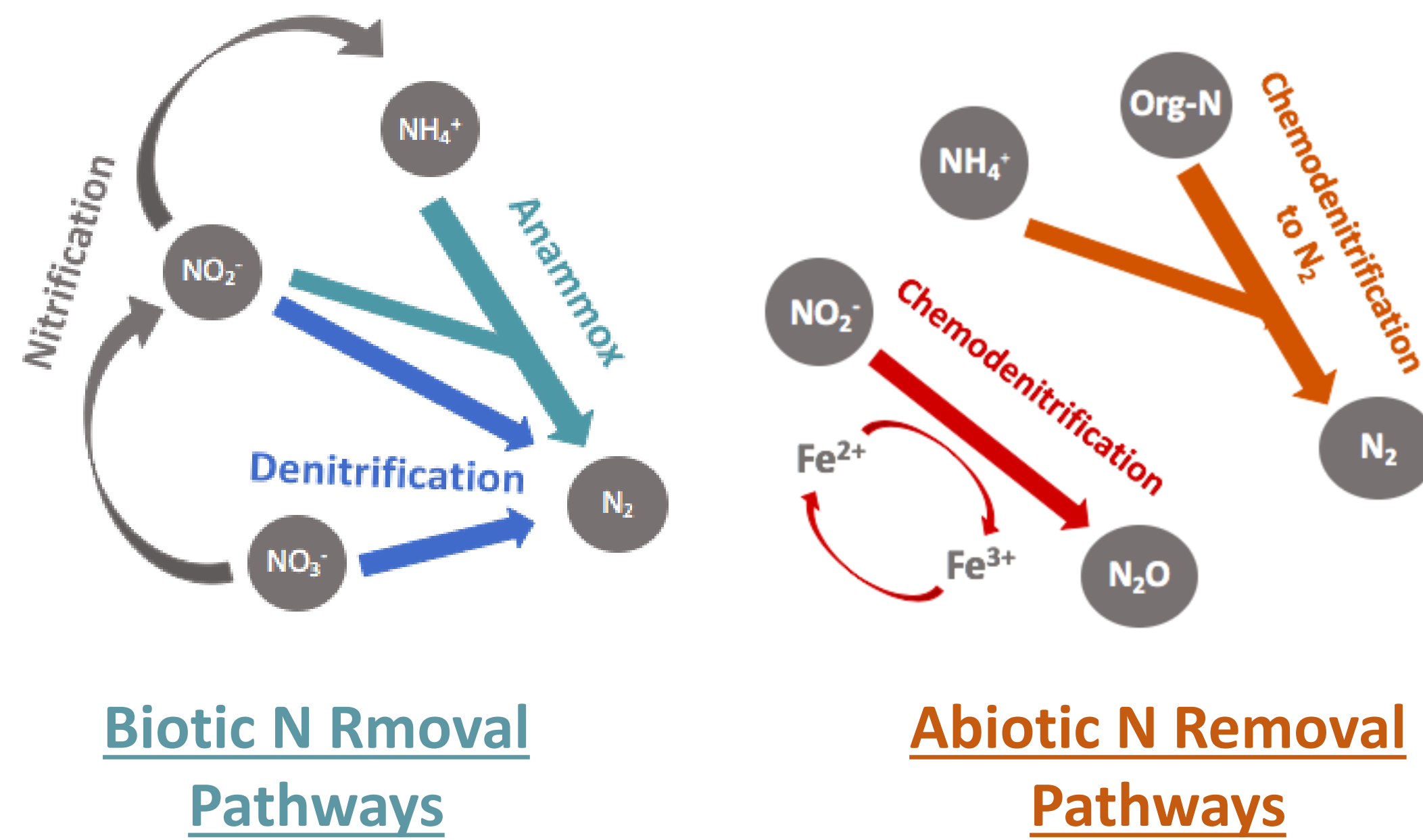


Denitrification and anammox are major enzymatic processes that effectively remove reactive nitrogen (N) in the environment by transforming it to inert dinitrogen (N₂) gas. Chemodenitrification is another N removal pathway in which nitrite (NO₂⁻) reacts abiotically with organic N or ammonium (NH₄⁺) to produce N₂. This reaction has been observed previously and chemodenitrification producing N₂O has been recognized in acidic soils, however, chemodenitrification to N₂ may also occur providing a new avenue of N removal^(1,2). This is an understudied pathway that calls into question assumptions in current models of the N cycle.

Research Objectives

- 1) Detect and quantify chemodenitrification to N₂ as an abiotic N removal pathway
- 2) Determine chemical characteristics affecting abiotic N₂ production
- 3) Evaluate the importance of abiotic N₂ production in the natural environment
- 4) Explore a pathway coupling biotic and abiotic processes of nitrogen removal



Methods

1. **Solution Incubations**
Sterile phosphate buffer pH adjusted amended with ¹⁵NO₂⁻ and ¹⁴N substrates
 2. **Sterile Soil/Sediment Incubations**
Soils and sediments of varying pH collected and sterilized for anoxic incubation with ¹⁵NO₂⁻
 3. **Abiotic – Biotic Coupling Incubation**
Live and dead soils from an acid mine drainage site incubated with ¹⁵NO₃⁻ and ¹⁵NO₂⁻
- ^{29,30}N₂ analysis by Isotope Ratio Mass Spectrometry

RESULTS:

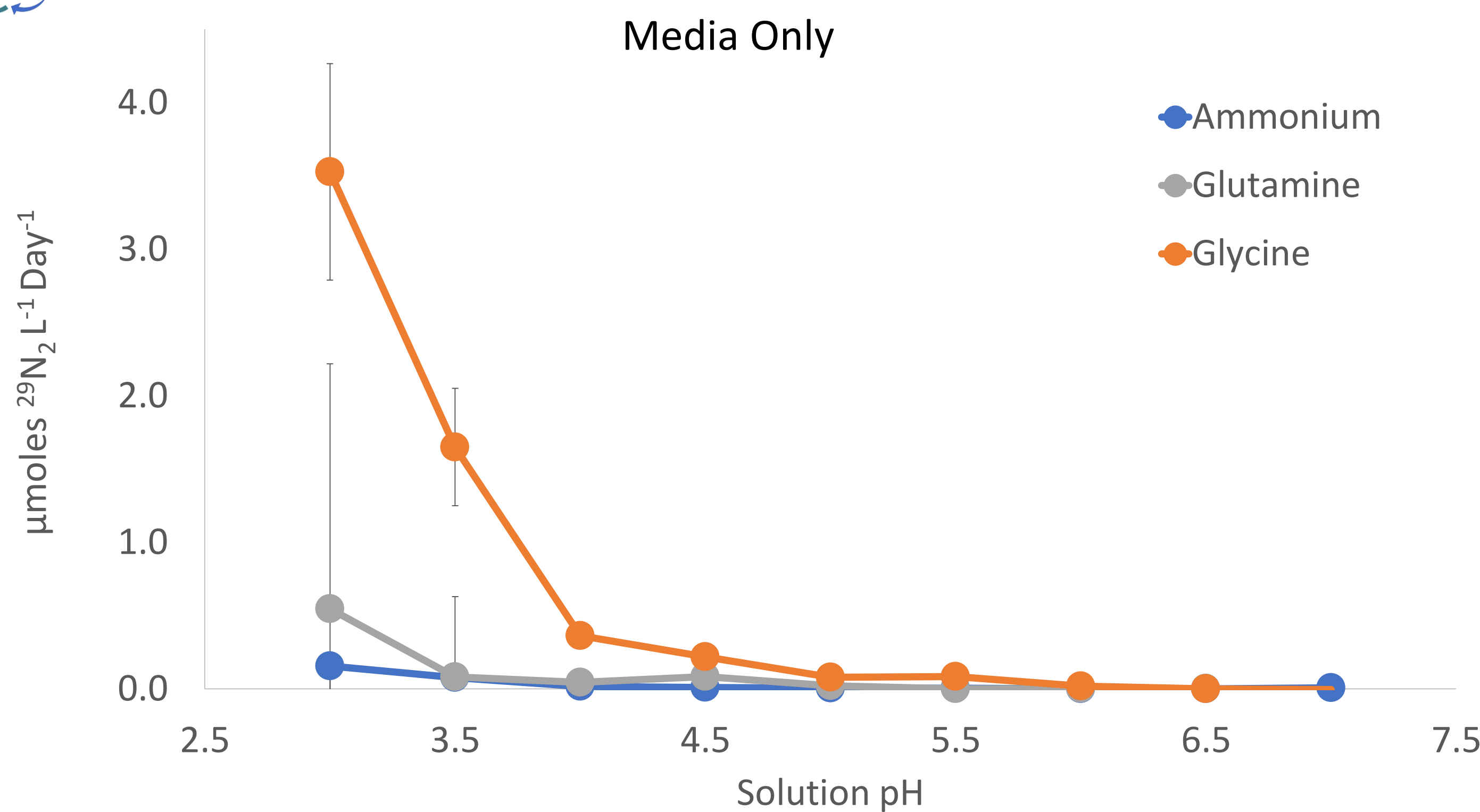


Figure 1. ²⁹N₂ production from ¹⁵NO₂⁻ and ¹⁴N-partners along a pH gradient with substrate concentrations of 1mM. The highest reactivity was observed at the lowest pH.

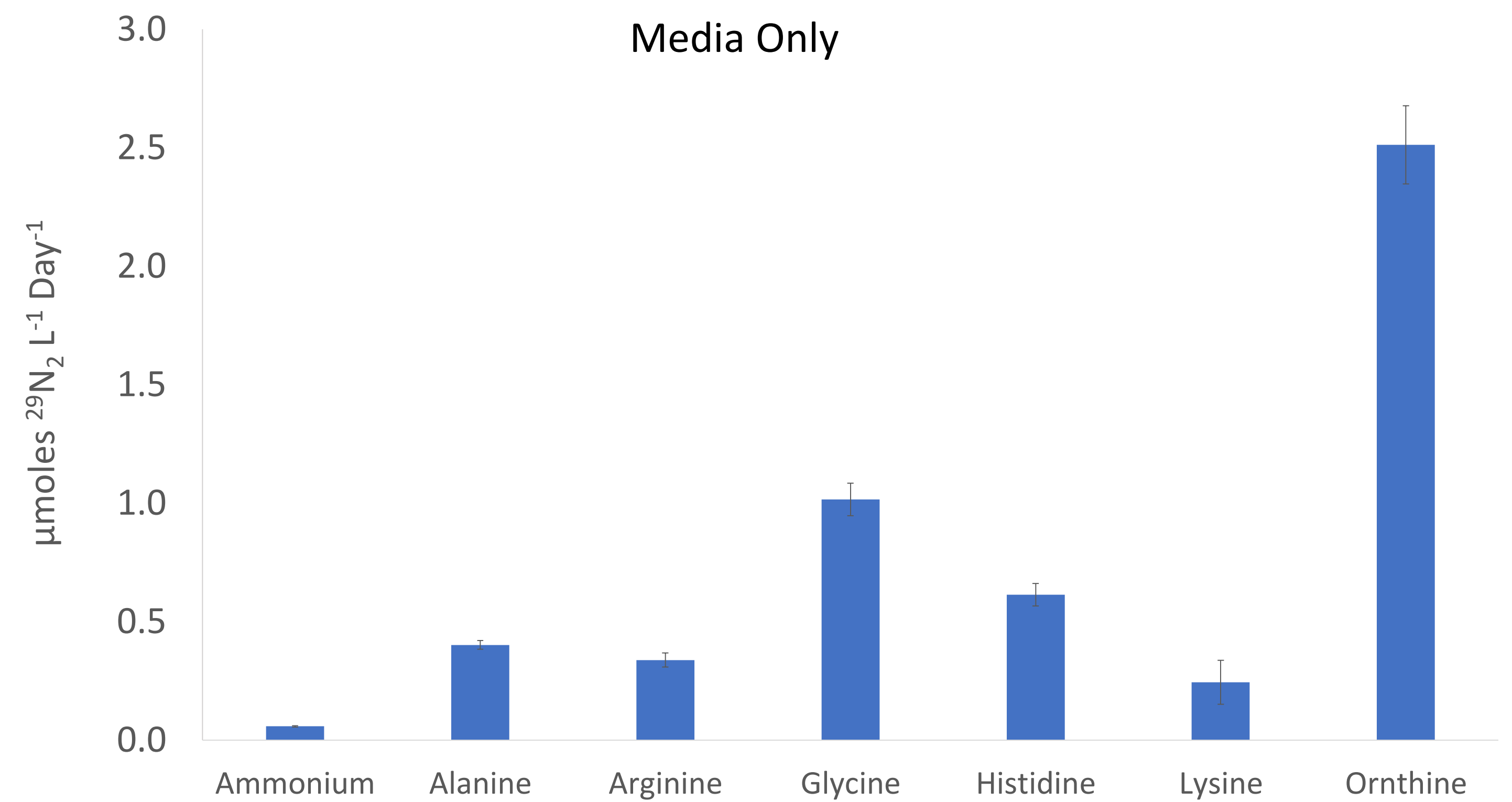


Figure 2. ²⁹N₂ production at pH 5 from ¹⁵NO₂⁻ and various ¹⁴N-partner amino acids found in soils with substrate concentrations of 5mM⁽³⁾. Differential reactivity indicates that some organic N is more reactive for abiotic N₂ production.

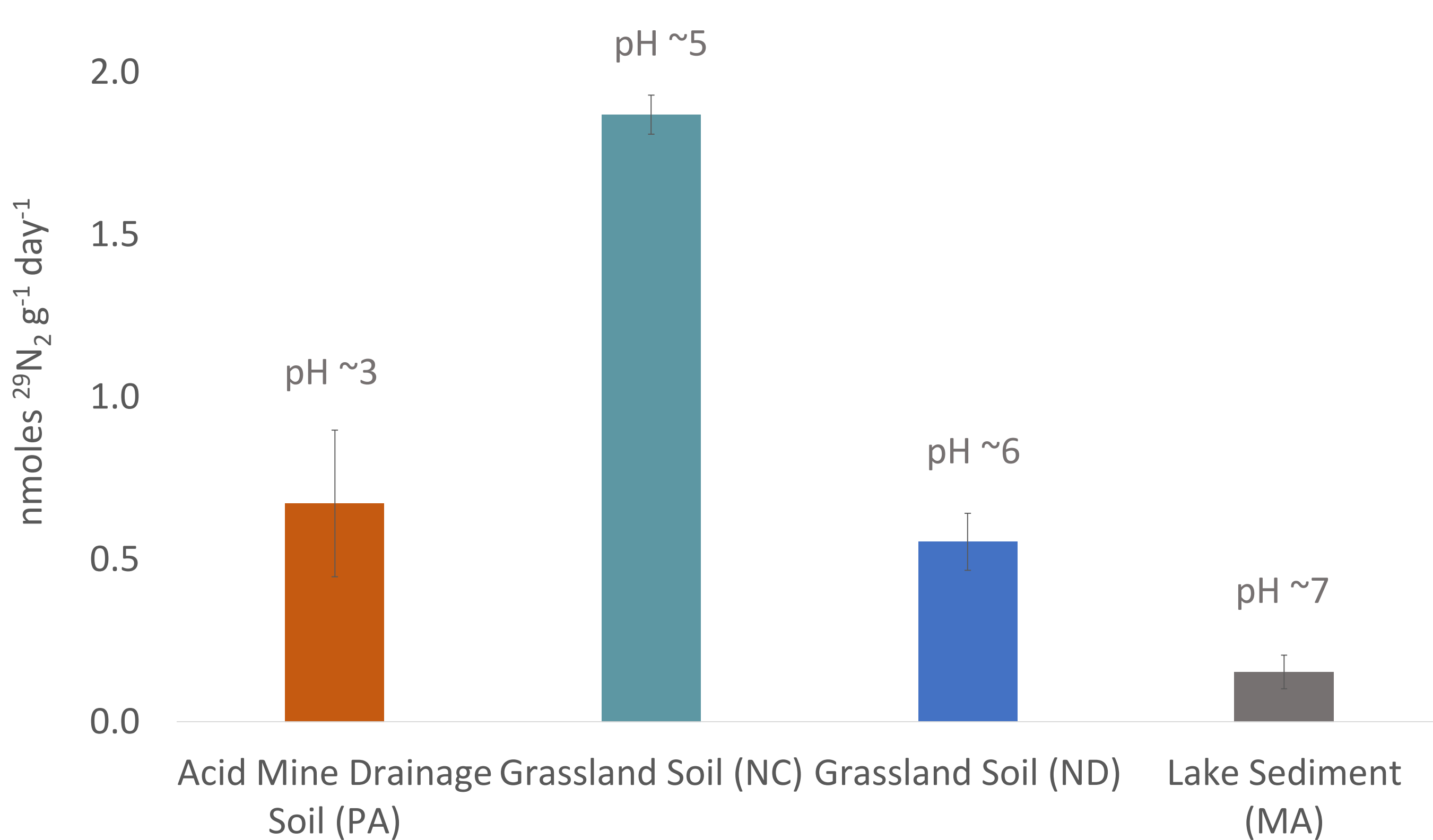


Figure 3. ²⁹N₂ production from ¹⁵NO₂⁻ amended soil samples of varying pH. Despite pH being lower in acid mine drainage soils the highest activity was observed in the NC grassland soils.

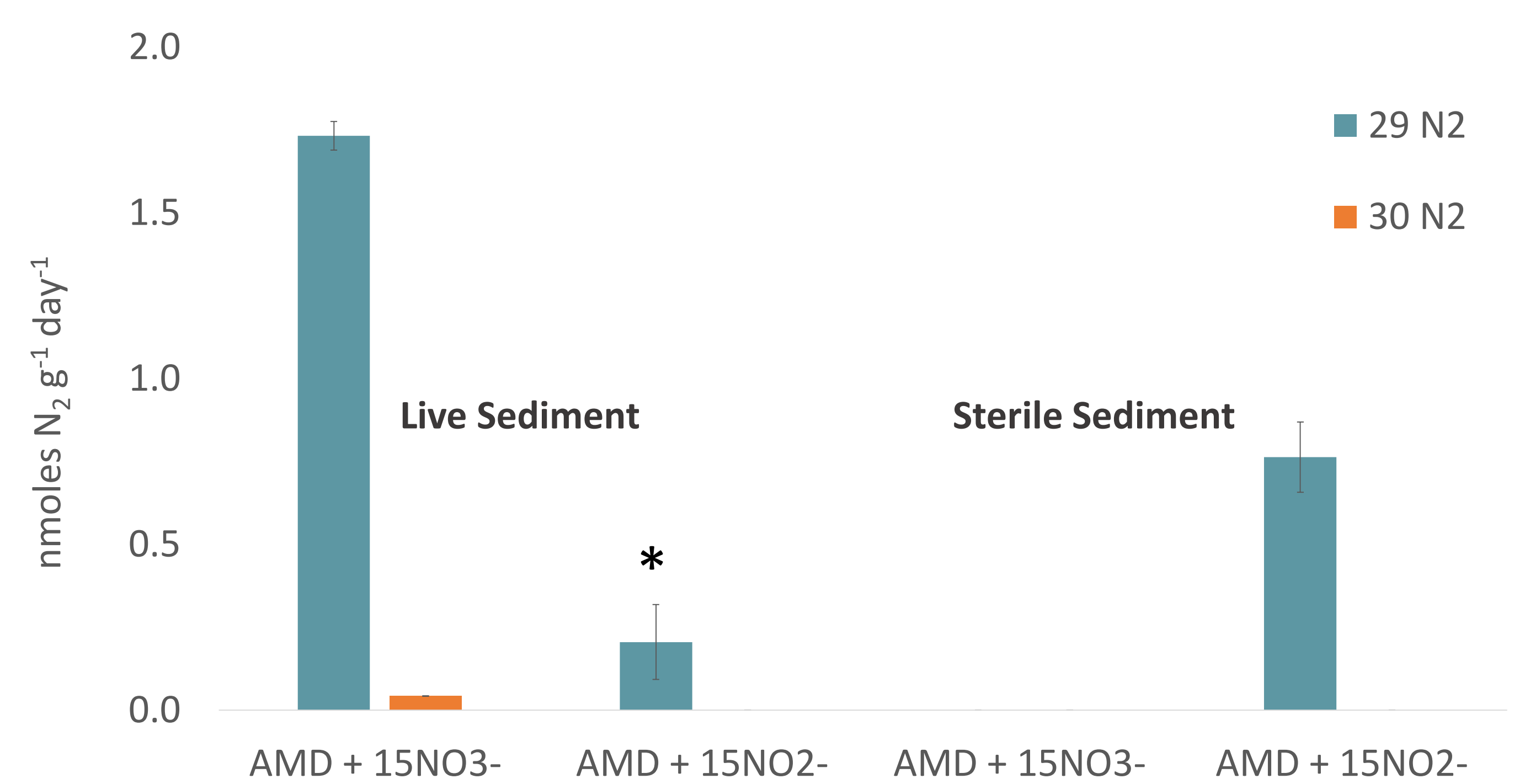


Figure 4. Comparison of denitrification (³⁰N₂) and chemodenitrification (²⁹N₂) in live and dead soils of acid mine drainage. Both ²⁹N₂ and ³⁰N₂ production was observed in the live soils with ¹⁵NO₃⁻ while ²⁹N₂ only was detected in the dead soils. * indicates abiotic ²⁹N₂ production in live soil samples.

CONCLUSIONS:

- Chemodenitrification to N₂ was detected in acidic solutions and all the soils/sediments tested.
- Both pH and organic content were identified as important characteristics affecting abiotic N₂ production.
- Abiotic N₂ production rate was highest in grassland soils of North Carolina, which may be associated with reactive organic N.
- A potential coupling of biotic reduction NO₃⁻ to NO₂⁻ and abiotic N₂ production was observed in acid mine drainage soils.

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