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Short Communication

Enhancement of subsoil denitrification using an electrode as an electron donor

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ABSTRACT

Laboratory culture studies have demonstrated that some microbial strains can use electrons generated by electrodes in the denitrification reaction. To test whether the native soil microbiota can use electrode electrons for denitrification, a subsoil slurry was incubated under an electric potential treatment. A potentiostat-poised (−500 mV) electrode served as an electron donor. The electric potential treatment enriches the electroactive denitrifying bacteria and accelerates the nitrate reduction in the subsoil slurry, with N_2 as the dominant end product. These results demonstrate that an electrode can serve as an electron donor to enhance the subsoil denitrification. This finding supports the future development of a technique to remove accumulated nitrate in subsoils and reduce nitrate contamination in groundwater.

The overuse of nitrogen (N) fertilizers can result in the accumulation of NO_3^- in the soil profile (Zhou et al., 2016). The accumulated NO_3^- in the subsoil may readily leach out and pollute groundwater (Peterson et al., 2013). The lack of an electron donor, e.g., dissolved organic carbon (DOC), is one of the key factors that limit the denitrification in subsoil (McCarty and Bremner, 1992). Consequently, introducing an electron donor (e.g., DOC) can enhance subsoil denitrification effectively (Qin et al., 2017). However, introducing DOC into the subsoil may be difficult and environmentally harmful because of the potential for secondary pollution of groundwater (Guggenberger and Kaiser, 2003; Hübner et al., 2012).

Some electroactive microbial strains, e.g., *Geobacter metallireducens* and *Pseudomonas alcaliphila*, was reported to use the electrons directly from an electrode at a low electric potential to reduce NO_3^- in bacterial cultures (Gregory et al., 2004; Su et al., 2012; Zhang et al., 2014a,b; Yu et al., 2015). These results indicate that the application of a low electric potential can readily provide donor electrons to enhance the subsoil denitrification. Until now, the use of electrode electrons for microbial denitrification has only been observed for a few microbial strains in laboratory culture studies. It remains unclear whether a native soil microbial consortium can use the electrons donated from an electrode to reduce NO_3^-.

We hypothesized that the application of a low electric potential would enrich the electroactive bacteria and promote denitrification in subsoil. The objective of this study was to test this hypothesis by investigating the effects of an applied electric potential on the denitrification rate, denitrification product composition and typical electroactive bacteria abundance in subsoil with high NO_3^- and low DOC concentrations (Supplemental Information).

Aliquots of fresh soil (5 g oven-dried basis) were added to 250-ml glass bottles. Then, 150 ml of distilled water and a magnetic stir bar were added to each bottle. A low soil/water ratio (1/30) was used to ensure the homogeneity of the suspension during incubation. The homogeneity was further enhanced by stirring at 125 rpm with a magnetic stir bar for 5 min. The slurry was stored at 4 °C before use.

The experiment had four treatments, each replicated three times: 1) fresh slurry with electric potential (F + E); 2) fresh slurry without electric potential (F-E); 3) autoclaved slurry with electric

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potential (A + E); 4) autoclaved slurry without electric potential (A-E). For treatment 1), 3 bottles were randomly selected from the prepared bottles. A graphite plate anode, a carbon brush cathode and a saturated calomel reference electrode were placed in each bottle. Then, the bottles were sealed using rubber septa and screw caps. Then, three thin metal wires were impaled through each septum and connected with the anode, cathode and reference electrode in the bottle (Fig. 1). To ensure the gas-tightness of the bottles, petrolatum was smeared on the joints among the septa, bottle and metal wires. The bottles were alternately evacuated (0.1 kPa) and filled with high-purity helium (99.9999%, 120 kPa) five times. The pressure of the headspace was adjusted to 101.3 kPa after the final filling with helium. The three iron wires were connected to a multi-channel potentiostat (CHI1040, Chenhua Co., Ltd., Shanghai, China) (Fig. 1). The effect of an applied electric potential on the microbial NO_3^- reduction increases with decreasing cathode potentials (Yu et al., 2015). Potentials higher than 600 mv are expected to produce H_2 via water electrolysis (Nevin et al., 2010). Thus, the cathode potential in this study was poised at −500 mV. The bottles were incubated with stirring at 125 rpm and 30 °C for 60 h.

Treatment 2 (F-E) was set up identically to treatment 1, except the electrodes were not connected to the potentiostat. Treatments 3 (A + E) and 4 (A-E) were set up identically to treatments 1 and 2, respectively, except the slurries were sterilized (autoclaved at 121 °C for 3 h and incubated at 25 °C for 21 h; three cycles) before use.

The headspaces of the bottles were periodically sampled and analysed for N_2O and N_2 concentrations using a robotized sampling and analysing system, which was described by Molstad et al. (2007). The slurry was simultaneously sampled with an injector to determine the NH_4^+, NO_3^- and DOC contents using the indophenol blue colorimetric method (Dorich and Nelson, 1983), dual-wavelength ultraviolet spectrophotometry (Norman et al., 1985) and TOC analyser (Shimadzu TOC-L, Japan) (Jones and Willett, 2006), respectively. The abundances of typical electroactive bacteria were determined via the 16s rRNA gene sequencing at the end of the incubation (Supplemental Information).

No significant emission of N_2 or N_2O was observed from the autoclaved slurries (Fig. 2), which indicates that the abiotic mechanisms did not generate N_2 and N_2O. However, the electric potential treatment significantly increased the production rate of N_2 and N_2O and decreased the NO_3^- concentration in the fresh soil slurry in comparison to the non-electric potential control (Fig. 2). The total amount of gaseous N that was produced as N_2 and N_2O (7.4 μmol N $bottle^{-1}$) was comparable to the amount of NO_3^- that was lost from the fresh slurry under the applied electric potential (6.9 μmol N $bottle^{-1}$) (Table 1). These results demonstrate that the electric potential significantly enhances the soil NO_3^- reduction via denitrification. Harmless N_2 was the dominant end product of denitrification in the electric potential treatment (Table 1), which is consistent with the previous study that applied an electric potential to groundwater (Zhang et al., 2014a).

Denitrification, dissimilatory NO_3^- reduction to NH_4^+ (DNRA), or both can account for the NO_3^- reduction with an electric potential depending on the variety of microbial strains (Gregory et al., 2004; Yu et al., 2015). Our results show that the electric potential treatment does not significantly affect the NH_4^+ concentration (Fig. 2), which indicates that DNRA did not occur. This result is consistent with a previous pure-strain study (Yu et al., 2015). The electric potential treatment significantly increased the relative abundance of *Geobacter* and *Pseudomonas* (Fig. 3). *Geobacter metallireducens* and *Pseudomonas alcaliphila* were reported to use electrode electrons for denitrification (Gregory et al., 2004; Su et al., 2012). Zhang et al. (2014a) also reported that the electric potential significantly enriched the denitrification bacteria content and promoted the denitrification rate in groundwater. These results demonstrate that the electrical potential treatment enhances the growth conditions

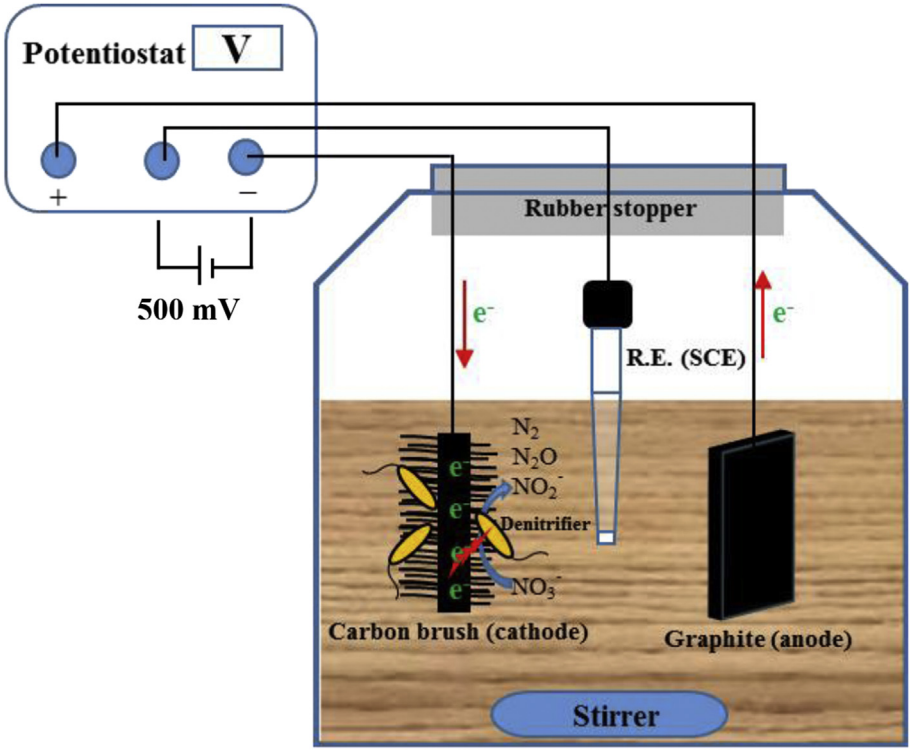

Fig. 1. Diagram of the incubating system. R.E. (SCE) refers to the reference electrode (saturated calomel electrode).