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EDITORIAL

Symbiotic nitrogen-fixing rhizobia as a potential source of nitrous oxide emissions

Los rizobios fijadores de nitrógeno como fuente potencial de emisiones de óxido nitroso

Nitrogen (N) is an indispensable nutrient for all forms of life, serving as an essential component of the main biomolecules. The N cycle, which is primarily driven by prokaryotic organisms, ensures the maintenance of a stable N balance within the biosphere. The entry point of N into this cycle is N fixation, a process in which dinitrogen (N_2) is enzymatically reduced to ammonium (NH_4^+) by nitrogenase, an enzyme exclusively produced by a specialized group of bacteria and archaea known as diazotrophs. Among the bacterial diazotrophs, rhizobia are considered the most agriculturally significant, forming symbiotic associations with legume plants, and several plant growth-promoting rhizobacteria (PGPR) capable of fixing N either in association with plant roots or as endophytes are also extensively used. Following their assimilation into biomass, NH_4^+ and its metabolic derivatives are released into the environment, where they undergo nitrification, a process that oxidizes these compounds into nitrate (NO_3^-). In turn, NO_3^- serves as a substrate for the denitrification process, which is carried out by a diversity of bacterial species.

Denitrification occurs under oxygen (O_2) limitation through a series of four redox reactions (Fig. 1), returning N_2 to the atmosphere and completing the nitrogen cycle. However, many bacterial species lack one or more genes encoding the enzymes necessary for a complete denitrification pathway, resulting in incomplete denitrification. For example, the absence of the *nosZ* gene can lead to the accumulation and release of nitrous oxide (N_2O), which is a potent greenhouse gas with significantly higher global warming potential than carbon dioxide (CO_2).

The emission of N_2O depends on the availability of nitrate (NO_3^-) as a substrate and is influenced by soil water content and organic matter levels[3]. Consequently, the use of N-fertilizers, which can leach NO_3^- into the soil, requires careful management. Excessive and unregulated fertilizer application, particularly without prior soil analysis and consideration of actual fertility needs, poses significant risks. This practice not only contributes to soil and water contamination, but also exacerbates greenhouse gas emissions. Alarmingly, such mismanagement has transformed agricultural soils into the largest anthropogenic source of N_2O emissions globally.

An alternative aimed to at least partially replace N-fertilizers is the use of N-fixing rhizobacteria. These bacteria are commercially utilized as inoculants for a variety of crops, where their application, either as PGPR or as symbiotic partners, significantly enhances plant growth and stress tolerance. Beyond their N-fixing capacity, these microorganisms increase the availability of other nutrients and synthesize phytohormones, which they release into the rhizosphere, thereby promoting plant growth and resilience. Rhizobia are a particularly important component of N-fixing species, which infect legume roots producing specialized structures called nodules, wherein rhizobia differentiate into specialized bacteroids with high N-fixing efficiency. This efficiency enables the rhizobia-legume symbiotic association to meet the complete N requirements of legumes, even when these plants are cultivated under hydroponic conditions in the absence of combined-N nutrients. However, some rhizobia can simultaneously function as denitrifiers, performing either the complete denitrification pathway to N_2 or truncated pathways ending in N_2O, depending on their genotypes. While rhizobial genera such as *Bradyrhizobium* and *Sinorhizobium/Ensifer* often encode at least part of the denitrification pathway, genera such as *Mesorhizobium* and *Rhizobium* rarely possess these genes[6]. The genus *Bradyrhizobium* is particularly significant as it includes species such as *B. diazoefficiens*, *B. elkanii*, and *B. japonicum*, which are widely used as inoculants for soybean cultivation. Furthermore, denitrification genes are widespread in *Bradyrhizobium* spp.; however, the complete denitrification pathway is present in only a small proportion of its species[2].

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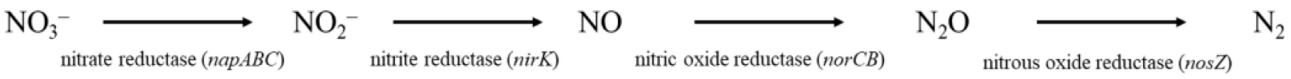

Figure 1 The denitrification pathway. Below each reaction, the corresponding enzyme names and the genes encoding them are indicated.

Soybean is the most important legume crop worldwide. Particularly, in Argentina, it covers more than 20 million hectares. A significant portion of these fields is inoculated with *Bradyrhizobium* spp. or nodulates with allochthonous soybean-nodulating rhizobia that persist in the soil from previous inoculations. Since these rhizobial species may perform denitrification in free-living state as well as in nodules[5], an inability of performing the complete denitrification pathway may result in N_2O emissions from the crop in this extensive cultivation area. In particular, it was observed that *B. japonicum* E109, which is widely used as soybean inoculant in Argentina, lacks the *nosZ* gene and therefore is a potential N_2O producer[4]. Conversely, inoculating soybean crops with a mixture of *nosZ*$^+$ *B. diazoefficiens* strains, isolated from local soils, has been shown to reduce N_2O emissions in field conditions[1]. This finding highlights the importance of developing selective inoculation strategies to mitigate greenhouse gas emissions from soybean cultivation.

Current DNA sequencing technologies have made available the complete genome sequences of numerous *Bradyrhizobium* species. However, only a small subset of these species carries all the genes required for a complete denitrification pathway. At the species level, solely *B. diazoefficiens* and *Bradyrhizobium oligotrophicum* species consistently harbor those genes in all strains sequenced to date, but only *B. diazoefficiens* is currently utilized in soybean inoculants. In contrast, members of the *B. elkanii* group and related strains typically contain only partial copies of the *nor* operon, rendering them incapable of completing the denitrification process. Additionally, most *B. japonicum* strains, as mentioned above for *B. japonicum* E109, lack the *nos* operon, which is essential for converting N_2O to N_2 (Fig. 1). As a result, these strains are more likely to contribute to N_2O emissions[4].

Therefore, soybean inoculation with *Bradyrhizobium* spp. can become a double-edged sword if the choice of species to inoculate is not carefully considered, and, in particular, if the presence of *nosZ* is not verified. Several PCR methods are available to determine the presence of *nosZ* in *Bradyrhizobium* spp.[2,4–6], and their simplicity and accuracy render these methods suitable for incorporation into routine quality control when new strains are selected for inoculants[2]. Reducing the prevalence of *nosZ*$^-$ strains in agricultural soils will require sustained efforts over time. Addressing this issue is urgent within the broader context of global initiatives aimed at mitigating greenhouse gas emissions. By prioritizing the use of *nosZ*$^+$ strains in inoculants, agricultural practices can contribute to both sustainable crop production and the reduction of the environmental impact of farming.

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