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Short Communication

A distinctive root-inhabiting denitrifying community with high $N_2O/(N_2O+N_2)$ product ratio

Chao Ai [a], Guoqing Liang [a], Xiubin Wang [a], Jingwen Sun [a], Ping He [a, b], Wei Zhou [a, *]

[a] Ministry of Agriculture Key Laboratory of Plant Nutrition and Fertilizer, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, 100081, PR China
[b] International Plant Nutrition Institute China Program, Beijing, 100081, PR China

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ABSTRACT

Microbial denitrification in agriculture makes a considerable contribution to terrestrial nitrous oxide (N_2O) emissions, and the prevailing view is that this mainly occurs in soil. Here, we show the root N_2O emission capacity of wheat grown under three long-term (32-year) fertilization regimes, and compare root-, rhizosphere- and soil-inhabiting denitrifying microbial communities. The $N_2O/(N_2O + N_2)$ product ratio of denitrification in the root was 0.5–9.2-fold higher than that in surrounding soil under fertilized conditions, especially manure application. Root $N_2O/(N_2O + N_2)$ ratio was closely related to the proportion of two nitrite-reductase genes (*nirK*/*nirS*), with higher N_2O emission associated with increased *nirS* abundance. Rhodobacterales and Pseudomonadales dominated the root-associated *nirS* community. In contrast, soils showed a higher proportion of unclassified denitrifiers. Our results demonstrate the potential of wheat to emit N_2O from the roots that harbour low-complexity denitrifying communities distinct from those occurred in soils.

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Agricultural fields with high nitrogen (N) application rates are thought to be hot spots for N_2O emissions and contribute about 60% (5.3 Tg N $year^{-1}$) of the total anthropogenic emissions and 30% of the total terrestrial emissions (Syakila and Kroeze, 2011). The established wisdom is that N_2O is produced mainly by soil-based microorganisms such as nitrifying and denitrifying bacteria; the latter reduce soluble nitrate (NO_3^-) or nitrite (NO_2^-) to dinitrogen (N_2) through a series of gaseous intermediates, including nitric oxide (NO) and nitrous oxide (N_2O). Recent studies have shown that living plants are a potentially important source of N_2O emissions in agricultural systems (Yu and Chen, 2009). For example, a wheat field study indicated that N_2O emissions increased with planting density and plant development (Zou et al., 2005). These studies propose three potential mechanisms for the plant emissions. Firstly, plant roots impact N_2O production in the rhizosphere soil where root exudates stimulate or inhibit microbial populations and their activities (Bardon et al., 2014; Henry et al., 2008; Sun et al., 2016). Second, plants can act as a conduit for transport of N_2O from the soil (Bowatte et al., 2014). Finally, N_2O emissions are directly detected in a particular plant organ or tissue. Recently, it was found that bacteria resident on plant leaves have the capacity to produce N_2O just as in soil (Bowatte et al., 2015). Similar to plant leaves, terrestrial plants harbour a root microbiome distinct from the complex microbial community present in surrounding soil (Bulgarelli et al., 2012; Lundberg et al., 2012). Surprisingly, a high number and diversity of denitrification genes have also been found inside plant roots by metagenomic analysis (Sessitsch et al., 2012). This might prompt us to ask whether the root-inhabiting denitrifiers have the capacity to contribute to terrestrial N_2O emissions as they do in soils.

Here we investigate the denitrification activity and N_2O emission potential of wheat root (*Triticum aestivum* L.), and compare root- and soil-inhabiting denitrifying microbial communities. We hypothesized that root had distinct denitrifying communities with different $N_2O/(N_2O + N_2)$ product ratio. In doing so, wheat plants were grown in fertilized soils of contrasting geochemistry, designated control (infertile), NPK (inorganic nutrient-rich) or MNPK (organic matter-rich) soil (Table 1). The root and soil samples were collected 47 days after planting, when plants were in an active vegetative growth state. The bulk soil was sampled from the root-free compartments (Fig. S1). The rhizosphere soil was obtained from the middle compartment of the rhizobox. Root cleaning

* Corresponding author. No.12, Zhongguancun South Street, Beijing, 100081, PR China.
E-mail address: wzhou@caas.ac.cn (W. Zhou).

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