Soil Biology and Biochemistry 119 (2018) 32–40

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Structure and assembly cues for rhizospheric *nirK*- and *nirS*-type denitrifier communities in long-term fertilized soils

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ARTICLE INFO

Keywords:
Denitrifier
nirS
nirK
Rhizosphere
Root-derived carbon
Stable isotope probing

ABSTRACT

The stimulatory effect of plants on soil denitrification activity has been widely reported, and root-derived carbon (C) is considered a factor contributing to enhanced denitrification activity in the rhizosphere. However, the mechanisms through which root-derived C shapes the rhizospheric denitrifying community structure remains unclear, especially under different soil fertility levels. Here, DNA-based stable isotope probing (DNA-SIP) and Illumina MiSeq sequencing were employed to characterize root-associated denitrifier communities containing nitrite reductase genes (*nirS* and *nirK*) in the rhizosphere of wheat grown in soils with three distinct long-term (32-year) fertilization regimes. Fertilization showed a significant impact on the composition of denitrifying communities that actively utilized photosynthetically fixed C in the wheat rhizosphere. In soils amended with inorganic fertilizers, wheat root-derived ^{13}C was mainly assimilated by *nirS*-type denitrifiers affiliated to Alcaligenaceae and *nirK*-type denitrifiers affiliated to Phyllobacteriaceae. In contrast, organic fertilization resulted in larger diversity of ^{13}C-labeled denitrifier communities where *nirS*-type denitrifiers such as Rhodobacteraceae and unclassified Burkholderiales and some unknown *nirK*-type denitrifiers were more abundant. The *nirS*-type denitrifier community was found to be more sensitive to the rhizosphere effect than the *nirK*-type community. Approximately 31% of the ^{13}C-labeled *nirS*-type denitrifiers were more abundant in the rhizosphere than in the bulk soil, but only 2% of the ^{13}C-labeled *nirK*-type denitrifiers showed increased abundance in the rhizosphere. The results of this study present direct evidence that root exudates can act as inducible C sources for heterotrophic denitrifying bacteria, but this induction pattern differs between *nirS*- and *nirK*-type communities and is dependent on soil fertility level.

1. Introduction

Nitrous oxide (N_2O) is a major stratospheric ozone-depleting substance and one of the most important greenhouse gases on Earth (Prentice et al., 2012; Blunden et al., 2013). The atmospheric concentration of N_2O has been increasing at nearly 0.75 ppb $year^{-1}$ since 1970 (IPCC, 2014). The increasing N_2O levels in the atmosphere are significantly associated with high nitrogen (N) and manure application rates in agricultural ecosystems (Syakila and Kroeze, 2011), and soil microbial denitrification plays a key role in this process. Denitrifying bacteria reduce soluble nitrate (NO_3^-) or nitrite (NO_2^-) to dinitrogen (N_2) through a series of gaseous intermediates, including nitric oxide (NO) and N_2O. Among these, the key reaction is reduction of NO_2^- to NO, which is catalyzed by two structurally different, but functionally equivalent NO_2^- reductases; namely, cytochrome cd1-containing reductase (NirS) and copper-containing reductase (NirK) (Hochstein and Tomlinson, 1988; Cutruzzola et al., 2001). This step causes dissolved N to become gaseous N for the first time during the denitrification process. Therefore, *nirS* and *nirK* genes are usually used as molecular markers to investigate the ecological behavior of denitrifying microorganisms in the environment. These two genes are thought to be mutually exclusive among denitrifying species and to represent two ecologically distinct denitrifying groups (Jones and Hallin, 2010). Several studies have shown that the responses of the *nirS*-type denitrifier community to environmental gradients are markedly different from those of the *nirK*-type denitrifier community, which supports the possibility that the two communities occupy different ecological niches (Jones and Hallin, 2010; Wei et al., 2015; Azziz et al., 2017).

Terrestrial plants release an array of substrates (i.e. rhizodeposition) that have the potential to lead to interactions between plants and

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https://doi.org/10.1016/j.soilbio.2018.01.007
Received 9 September 2017; Received in revised form 7 January 2018; Accepted 10 January 2018