Soil Biology & Biochemistry 109 (2017) 118–123

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

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

ARTICLE INFO

Article history:
Received 4 September 2016
Received in revised form
19 January 2017
Accepted 10 February 2017

Keywords:
Denitrification
Fertilization
Nitrous oxide
Rhizosphere
Root

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.

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).

http://dx.doi.org/10.1016/j.soilbio.2017.02.008
0038-0717/

Table 1
Root and soil chemical characteristics. Data are means ± SEM. Fertilization treatments: Control, non-fertilizer; NPK, inorganic N, P, and K fertilizer; MNPK, manure plus NPK fertilizer.

Treatments	Fractions	pH	Total C (g kg^{-1})	Total N (g kg^{-1})	C/N ratio	DOC (mg kg^{-1})	DON (mg kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})
Root	Control	–	418.58 ± 0.88	8.21 ± 0.11	50.96 ± 0.61	–	–	495.51 ± 55.05	363.33 ± 40.43
	NPK	–	405.76 ± 2.41	9.49 ± 0.44	42.83 ± 2.21	–	–	627.43 ± 40.69	419.2 ± 62.17
	MNPK	–	370.58 ± 2.49	11.7 ± 0.64	31.74 ± 1.54	–	–	843.25 ± 34.53	489.81 ± 35.21
Rhizosphere	Control	8.12 ± 0.02	7.39 ± 0.14	0.84 ± 0.03	8.8 ± 0.17	49.89 ± 1.58	14.81 ± 0.79	2.97 ± 0.18	3.51 ± 0.12
	NPK	8.04 ± 0.02	9.09 ± 0.05	1.07 ± 0.03	8.52 ± 0.18	65.19 ± 4.5	21.62 ± 1.01	2.79 ± 0.11	7.46 ± 0.36
	MNPK	7.78 ± 0.02	16.28 ± 0.43	1.77 ± 0.12	9.24 ± 0.5	90.41 ± 8.14	28.76 ± 1.32	4.22 ± 0.35	10.4 ± 0.19
Bulk soil	Control	8.28 ± 0.03	7.28 ± 0.17	0.84 ± 0.03	8.64 ± 0.49	39.22 ± 4.6	16.6 ± 0.6	3.06 ± 0.56	5.38 ± 0.51
	NPK	8.05 ± 0.02	8.18 ± 0.08	0.93 ± 0.03	8.81 ± 0.17	34.73 ± 1.84	16.47 ± 0.59	4.51 ± 0.22	5.82 ± 0.24
	MNPK	7.82 ± 0.03	16.92 ± 0.15	1.71 ± 0.04	9.91 ± 0.27	56.28 ± 3.56	28.52 ± 0.21	12.07 ± 1.06	8.67 ± 0.5

procedure were performed in strict accordance with the method recommended by Lundberg et al. (2012). When tiny soil aggregates were disrupted and cleaned by vortexing and sonication, clean roots were either immediately (within 2 h) used to determine the total denitrification activity and potential N_2O emission, or frozen with liquid nitrogen, freeze-dried and then stored at −80 °C until molecular analyses. More details are presented in Supplementary Information.

To determine whether root-inhabiting denitrifiers could produce N_2O, we calculated potential N_2O emission and total denitrification activity of sonication-cleaned root in anaerobic microcosms without and with acetylene, respectively (Fig. S2). The activity of denitrifying communities varied substantially across the different compartments, with values of 72.32–1662.83 and 11.76–75.99 ng N_2O-N g^{-1} (root or soil) dry weight h^{-1} for total denitrification activity and potential N_2O emissions, respectively. Organic treatment strongly enhanced the total denitrification activity by 9-fold in the bulk soil. The proportion of N_2O emitted by denitrification, calculated as the ratio of the rate of potential N_2O production and total denitrification activity ($N_2O/(N_2O + N_2)$) (Jones et al., 2014), was 1.2-fold higher in the root than in rhizosphere soil under no fertilization conditions (Fig. 1). The application of inorganic and organic manure led to a further 0.9–1.4-fold increase in root $N_2O/(N_2O + N_2)$ compared with control, whereas decreasing trends were observed in rhizosphere and bulk soil when organic manure was applied.

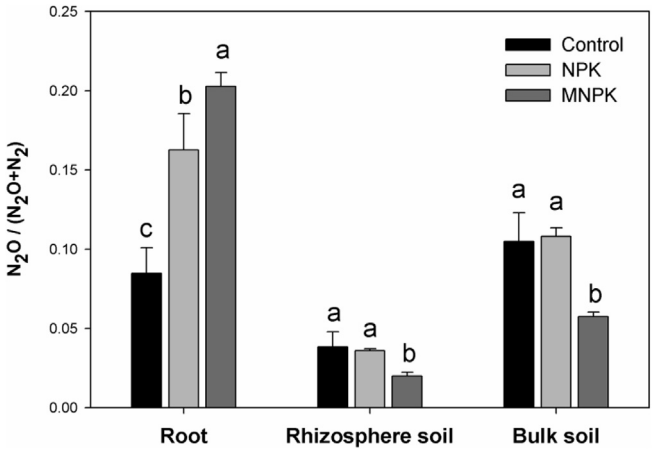

Fig. 1. High proportion of N_2O emitted by denitrification in the root. $N_2O/(N_2O + N_2)$ is calculated as the ratio of the rate of potential N_2O emission and total denitrification activity (Fig. S2). The values given are the means ± SEM of three separate treatments and different letters indicate significant differences among fertilizer treatments at $p < 0.05$, Fisher's LSD test. Fertilization treatments: control, no fertilizer; NPK, inorganic N, P, and K fertilizer; MNPK, manure plus NPK fertilizer.

The abundance of organisms with potential to produce N_2O by denitrification were determined by quantitative real-time PCR of the *nirK* and *nirS* genes that encode two structurally different but functionally equivalent nitrite reductases (Wei et al., 2015). Across the different fertilization treatments, the abundances of *nirK* and *nirS* genes in the root ranged from 0.5 to 9.3 × 10^9 copies g^{-1} root dry weight, with *nirK* being 1.1–11.6 times more abundant than *nirS* (Fig. 2A and B). Fertilization practices, especially manure application, substantially increased root *nirS* gene abundance, resulting in distinctively different root *nirK*/*nirS* ratios of approximately 12.6, 6.8 and 2.1 in the control, NPK and MNPK treatments, respectively (Fig. 2C). In contrast, rhizosphere and bulk soils had lower ratios of *nirK* to *nirS* than the root in the control and NPK treatments, and no significant differences were observed among the different fertilization treatments. Microorganisms with the capacity to reduced N_2O were quantified by targeting the nitrous oxide reductase gene (*nosZ*), whose abundance was markedly increased in root by the application of inorganic and organic fertilizers (Fig. 2D). The ratio of total *nir* genes to *nosZ* in root ranged from 8.3 to 10.5 and was statistically significantly higher than that in rhizosphere and bulk soil ($P < 0.05$).

Multiple stepwise regression analysis indicated that high *nirS* abundance and low *nirK*/*nirS* ratio best explained the increase in root $N_2O/(N_2O + N_2)$ ratio (Table 2), suggesting that the functional predominance of *nirS*-type denitrifiers in root might have substantial consequences for root N_2O production capacity. In contrast, the abundance of the *nosZ* gene in rhizosphere and bulk soil was the key and robust predictor that explained 79–82% of the variation in $N_2O/(N_2O + N_2)$ ratio (Table 2; Fig. S3).

When finding root N_2O emission potential was strongly associated with the proportion of *nirK*- and *nirS*-type denitrifiers, we analysed the diversity of *nirK* and *nirS* genes by Illumina MiSeq sequencing. Compared with rhizosphere and bulk soils, the *nirS*-type communities in root showed significantly less diversity by both Shannon index and Invsimpson index (Fig. S4), but no significant differences in *nirK* diversity were obtained. We constructed two reference libraries of *nirK*- and *nirS*-type strain sequences retrieved from all draft and completed microbial genomes obtained from NCBI. These known sequences served as references within the phylogenetic pplacer program (Matsen et al., 2010) to classify approximately 41–50% of root *nirK* and *nirS* sequences to species level (i.e., terminal edges of the reference phylogeny; Figs. 3A and 4A). Order-level (Fig. 3B, C and D) and genus-level (Fig. S5) identification of these sequences revealed a specific composition of the *nirS*-type communities in the root. Pseudomonadales and Rhodobacterales were the dominant orders (from 19 to 76%) in the root, especially when organic manure was applied (Fig. 3B), whereas Rhizobiales (14–20%) predominated in rhizosphere and bulk soil

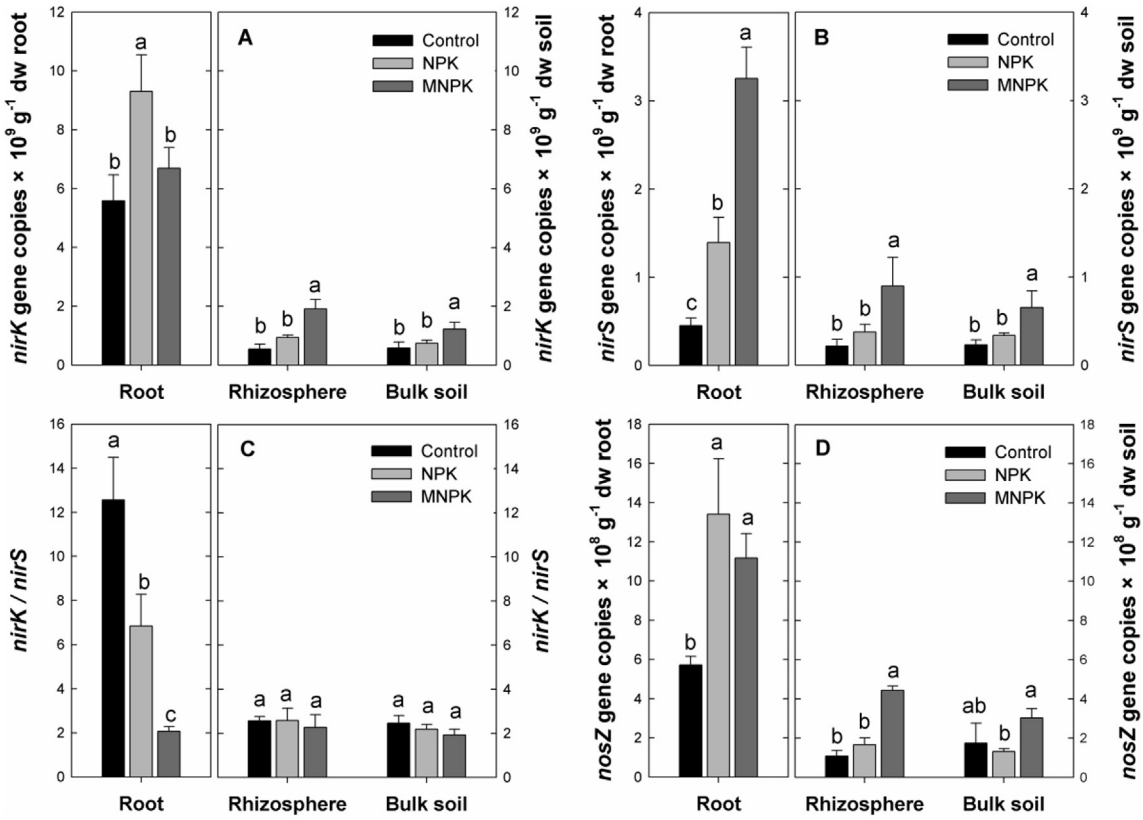

Fig. 2. Abundance of denitrification genes in the root. Effect of fertilization treatments on (A) *nirK* abundance, (B) *nirS* abundance, (C) *nirK*/*nirS* ratio and (D) *nosZ* abundance. The values given are the means ± SEM of three separate treatments and different letters indicate significant differences among fertilizer treatments at $p < 0.05$, Fisher's LSD test. Control, no fertilizer; NPK, inorganic N, P, and K fertilizer; MNPK, manure plus NPK fertilizer; dw, dry weight.

Table 2
Stepwise regressions to identify abundance of denitrifying genes making the strongest statistical contributions to variation in the $N_2O/(N_2O + N_2)$ ratio. Independent variables include the copy numbers of *nirK*, *nirS* and *nosZ* genes, the sum of *nirK* and *nirS* gene copies (*nirK* + *nirS*), and the ratio of the sum of two *nir* genes copies to the *nosZ* gene copies ((*nirK* + *nirS*)/*nosZ*). Dependents variable is $N_2O/(N_2O + N_2)$ product ratio.

Dependents	Variables related	R^2 (model)	F (model)	P (model)	Variables removed
Root	*nirS*, *nirK*/*nirS*	0.82	32.38	<0.001	*nirK*, *nosZ*, *nirK* + *nirS*, (*nirK* + *nirS*)/*nosZ*
Rhizosphere	*nosZ*	0.83	33.04	<0.001	*nirK*, *nirS*, *nirK*/*nirS*, *nirK* + *nirS*, (*nirK* + *nirS*)/*nosZ*
Bulk soil	*nosZ*	0.82	32.90	<0.001	*nirK*, *nirS*, *nirK*/*nirS*, *nirK* + *nirS*, (*nirK* + *nirS*)/*nosZ*

(Fig. 3C and D). In addition, approximately 64–70% of the sequences in rhizosphere and bulk soil mapped to deeper internal edges of the reference phylogeny (i.e. unclassified groups in Fig. 3C and D), indicating that there remains a large degree of *nirS* diversity that is not represented in the present genomic database. In contrast to *nirS*-type denitrifiers, the majority of *nirK*-type denitrifiers (22–65%) were similar to Rhizobiales (Fig. 4B, C and D and Fig. S6), whose abundance was not statistically significantly different (Two-way ANOVA, $P < 0.05$) among the different compartments (root, rhizosphere and bulk soil).

The stimulatory effect of the plant on soil denitrification has been widely reported (Bodelier et al., 1997; Qian et al., 1997), and root exudates and conduit effect are considered as key determinants of enhanced denitrification activity and N_2O emission in the rhizosphere (Bowatte et al., 2014; Sun et al., 2016). In this study, the total denitrification activity was shown to be significantly higher in wheat rhizosphere than in the bulk soil (Fig. S2), confirming previous observations (Bodelier et al., 1997; Henry et al., 2008; Prade and Trolldenier, 1988). Further, we found that the $N_2O/(N_2O + N_2)$ ratio in the root compartment was 1.2–9.2-fold higher than in rhizosphere soil, especially under organic fertilization, implying that root-inhabiting microbes themselves also have the capacity to produce N_2O just as in soil when the condition is met. When the soil was rich in organic matter, Prade and Trolldenier (1988) found that root-dependent respiration would amplify O_2 depletion and accelerate the onset of denitrification in planted soil. Recently, it has been reported that N_2O emissions from nodulated soybean roots were increased 830-fold under flooding and high N conditions (Tortosa et al., 2015). These results suggest that the aeration status of root zone may be a key factor controlling root denitrification activity and consequently N_2O emissions.

In this work, *nirS*-type rather than *nirK*-type denitrifiers functionally dominate the root reduction of NO_2^- to N_2O, even though the latter were on average 3-fold higher in abundance than the

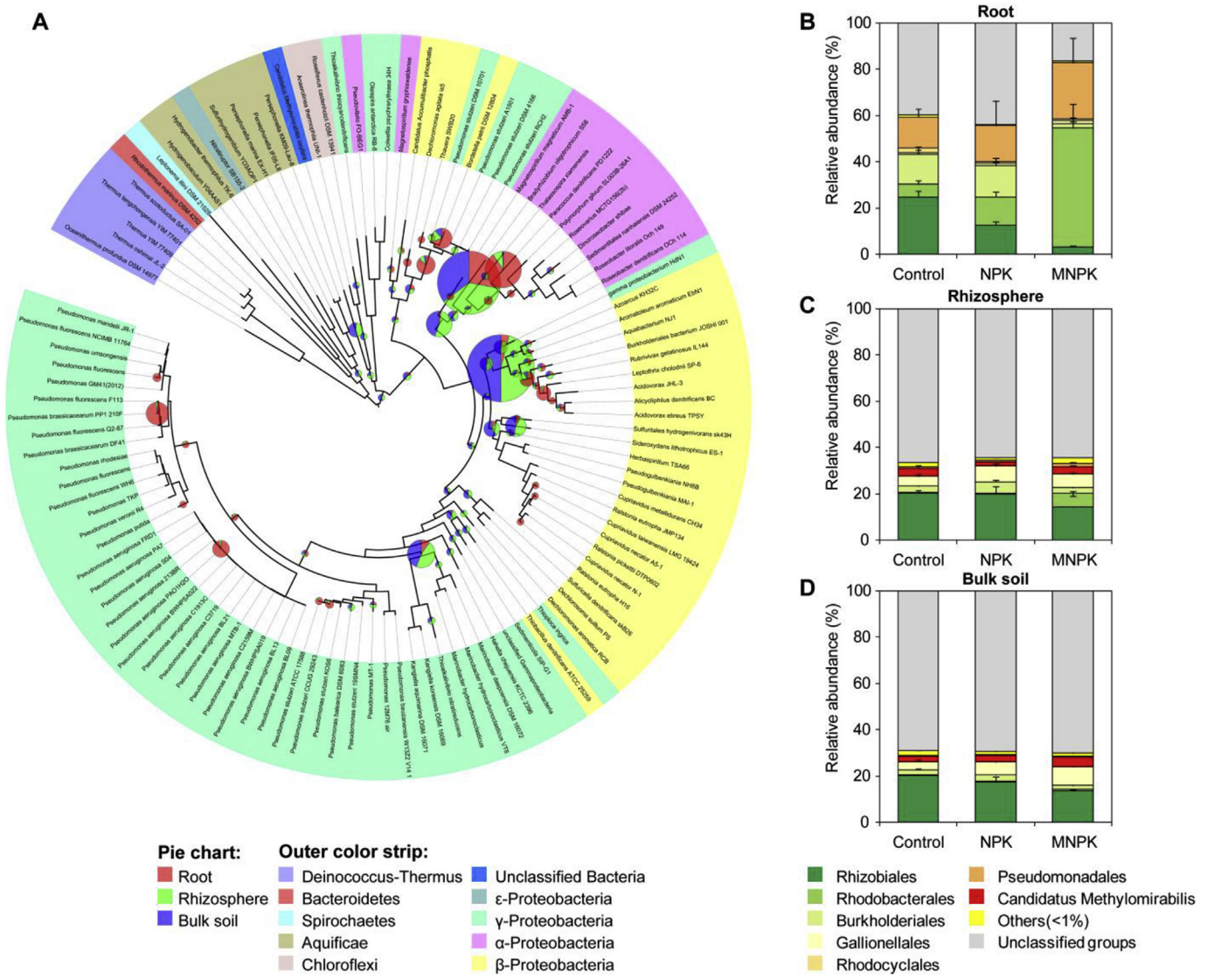

Fig. 3. Phylogenetic placement of *nirS* sequencing reads within a reference phylogeny. (A) The phylogeny was generated by using maximum-likelihood analyses of full-length *nirS* amino acid sequences obtained from known microbial genomes. Pie charts within the phylogeny represent the distribution of reads in the root, rhizosphere and bulk soil, and their placements are determined by the pplacer algorithm (Matsen et al., 2010). The outer colour strip shows the phylum distributions of reference strains. (B–D) Taxonomic composition (at the order level) of the *nirS* reads mapped to terminal edges of the reference phylogeny in root (B), rhizosphere (C) and bulk soil (D). The *nirS* reads mapped to deeper internal edges of the phylogeny are defined as 'unclassified groups', indicating that there remains a large degree of *nirS* diversity that is not represented in the current genome database. Fertilization treatments: control, no fertilizer; NPK, inorganic N, P, and K fertilizer; MNPK, manure plus NPK fertilizer.

former. The *nirS*-type denitrifiers have been previously reported to be more associated with vegetation than the *nirK*-type denitrifiers (Guo et al., 2013). Our results showed that the *nirS*-type denitrifiers of Pseudomonadaceae and Rhodobacterales, notably *Paracoccus denitrificans*, were strongly enriched in the organic manure-treated root (accounting for nearly 76% of root *nirS*-type denitrifiers). *Paracoccus denitrificans* is a model organism for denitrification study (Sears et al., 1997) and its presence has been confirmed in a number of plant roots (de Carvalho Costa and de Melo, 2012; Tsavkelova et al., 2007). Moreover, evidence is provided for both anaerobic and aerobic denitrification by this bacterium, with higher N_2O production and lower N_2 production in the presence of O_2 (Davies et al., 1989).

In conclusion, our results show that root itself also has the potential to contribute to the process of denitrification, as evidenced by the high $N_2O/(N_2O + N_2)$ product ratio and the distinct denitrifying communities in the wheat root. Although we cannot rule out that the root-associated N_2O production may be further transformed or consumed in the rhizosphere, abundant denitrifying genes found in wheat root support our argument that root can be potential 'plant' source of N_2O. It is worth noting that our wheat roots were collected using a sonication-dependent procedure (Bulgarelli et al., 2012; Lundberg et al., 2012), which may fail to release some rhizoplane microbes that live in a microniche shielded from the ultrasound (Lundberg et al., 2012). Thus, root-inhabiting denitrifiers in this study may differ slightly from classic definitions of endophytes that rely on partitioning culturable bacteria. Further study is needed to partition and quantify the actual contribution of root-endophytic, rhizosphere and bulk soil microbes to total terrestrial N_2O emissions under different soil conditions.

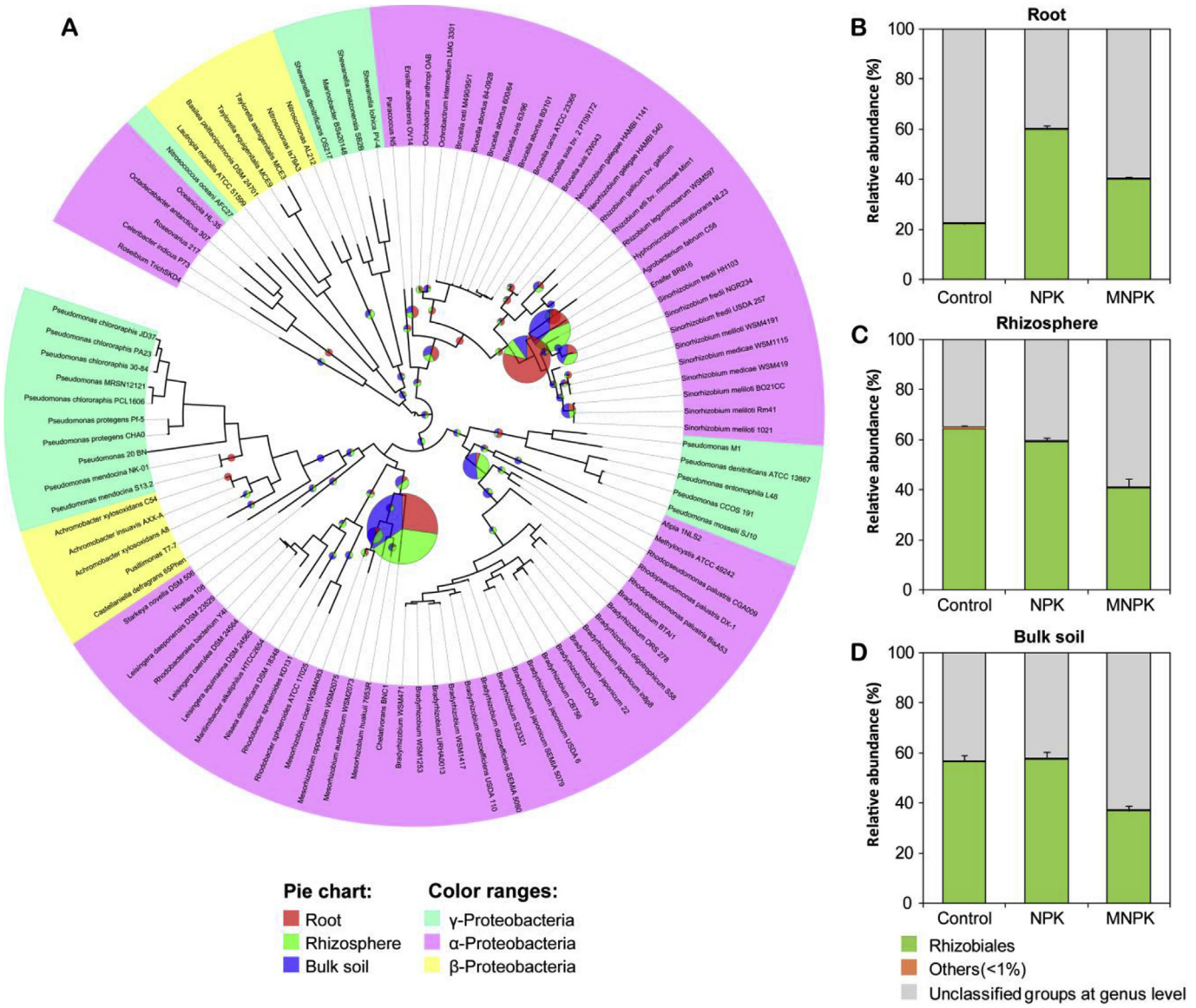

Fig. 4. Phylogenetic placement of *nirK* sequencing reads within a reference phylogeny. (A) The phylogeny was generated by using maximum-likelihood analyses of full-length *nirK* amino acid sequences obtained from known microbial genomes. Pie charts within the phylogeny represent the distribution of reads in the root, rhizosphere and bulk soil, and their placements are determined by the pplacer algorithm (Matsen et al., 2010). The outer colour strip shows the phylum distributions of reference strains. (B–D) Taxonomic composition (at the order level) of the *nirK* reads mapped to terminal edges of the reference phylogeny in root (B), rhizosphere (C) and bulk soil (D). The *nirK* reads mapped to deeper internal edges of the phylogeny are defined as 'unclassified groups', indicating that there remains a large degree of *nirK* diversity that is not represented in the current genome database. Fertilization treatments: control, no fertilizer; NPK, inorganic N, P, and K fertilizer; MNPK, manure plus NPK fertilizer.

Acknowledgments

We thank assistances from Qinlei Rong in field sampling, from Qian Zhang in soil chemical analyses, and from Peng Xu in microbial and functional gene analyses. We are grateful to Evgenia Blagodatskaya and five anonymous reviewers for their constructive comments and suggestions on this manuscript. This research was supported by the National Natural Science Foundation of China (31372136) and the National Basic Research Program of China (2013CB127405).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.02.008.

References

Bardon, C., Piola, F., Bellvert, F., Haichar, F.e.Z., Comte, G., Meiffren, G., Pommier, T., Puijalon, S., Tsafack, N., Poly, F., 2014. Evidence for biological denitrification inhibition (BDI) by plant secondary metabolites. New Phytologist 204, 620–630.

Bodelier, P.L., Wijlhuizen, A.G., Blom, C.W., Laanbroek, H.J., 1997. Effects of photoperiod on growth of and denitrification by Pseudomonas chlororaphis in the root zone of Glyceria maxima, studied in a gnotobiotic microcosm. Plant and Soil 190, 91–103.

Bowatte, S., Newton, P.C., Brock, S., Theobald, P., Luo, D., 2015. Bacteria on leaves: a previously unrecognised source of N_2O in grazed pastures. The ISME Journal 9, 265–267.

Bowatte, S., Newton, P.D., Theobald, P., Brock, S., Hunt, C., Lieffering, M., Sevier, S., Gebbie, S., Luo, D., 2014. Emissions of nitrous oxide from the leaves of grasses. Plant and Soil 374, 275–283.

Bulgarelli, D., Rott, M., Schlaeppi, K., van Themaat, E.V.L., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O., Amann, R., Eickhorst, T., Schulze-Lefert, P., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial

microbiota. Nature 488, 91–95.
Davies, K.J., Lloyd, D., Boddy, L., 1989. The effect of oxygen on denitrification in Paracoccus denitrificans and Pseudomonas aeruginosa. Journal of general microbiology 135, 2445–2451.
de Carvalho Costa, F.E., de Melo, I.S., 2012. Endophytic and rhizospheric bacteria from Opuntia ficus-indica mill and their ability to promote plant growth in cowpea, Vigna unguiculata (L.) Walp. African Journal of Microbiology Research 6, 1345–1353.
Guo, G.X., Deng, H., Qiao, M., Yao, H.Y., Zhu, Y.G., 2013. Effect of long-term wastewater irrigation on potential denitrification and denitrifying communities in soils at the watershed scale. Environmental Science & Technology 47, 3105–3113.
Henry, S., Texier, S., Hallet, S., Bru, D., Dambreville, C., Chèneby, D., Bizouard, F., Germon, J.C., Philippot, L., 2008. Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: insight into the role of root exudates. Environmental Microbiology 10, 3082–3092.
Jones, C.M., Spor, A., Brennan, F.P., Breuil, M.-C., Bru, D., Lemanceau, P., Griffiths, B., Hallin, S., Philippot, L., 2014. Recently identified microbial guild mediates soil N_2O sink capacity. Nature Climate Change 4, 801–805.
Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., del Rio, T.G., Edgar, R.C., Eickhorst, T., Ley, R.E., Hugenholtz, P., Tringe, S.G., Dangl, J.L., 2012. Defining the core Arabidopsis thaliana root microbiome. Nature 488, 86–90.
Matsen, F.A., Kodner, R.B., Armbrust, E.V., 2010. pplacer: linear time maximum-likelihood and Bayesian phylogenetic placement of sequences onto a fixed reference tree. BMC Bioinformatics 11, 538.
Prade, K., Trolldenier, G., 1988. Effect of wheat roots on denitrification at varying soil air-filled porosity and organic-carbon content. Biology and Fertility of Soils 7, 1–6.
Qian, J.H., Doran, J.W., Walters, D.T., 1997. Maize plant contributions to root zone available carbon and microbial transformations of nitrogen. Soil Biology and Biochemistry 29, 1451–1462.
Sears, H.J., Spiro, S., Richardson, D.J., 1997. Effect of carbon substrate and aeration on nitrate reduction and expression of the periplasmic and membrane-bound nitrate reductases in carbon-limited continuous cultures of *Paracoccus denitrificans* Pd1222. Microbiology 143, 3767–3774.
Sessitsch, A., Hardoim, P., Döring, J., Weilharter, A., Krause, A., Woyke, T., Mitter, B., Hauberg-Lotte, L., Friedrich, F., Rahalkar, M., 2012. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. Molecular Plant-Microbe Interactions 25, 28–36.
Sun, L., Lu, Y., Kronzucker, H.J., Shi, W., 2016. Quantification and enzyme targets of fatty acid amides from duckweed root exudates involved in the stimulation of denitrification. Journal of Plant Physiology 198, 81–88.
Syakila, A., Kroeze, C., 2011. The global nitrous oxide budget revisited. Greenhouse Gas Measurement and Management 1, 17–26.
Tortosa, G., Hidalgo, A., Salas, A., Bedmar, E.J., Mesa, S., Delgado, M.J., 2015. Nitrate and flooding induce N2O emissions from soybean nodules. Symbiosis 67, 125–133.
Tsavkelova, E.A., Cherdyntseva, T.A., Botina, S.G., Netrusov, A.I., 2007. Bacteria associated with orchid roots and microbial production of auxin. Microbiological Research 162, 69–76.
Wei, W., Isobe, K., Nishizawa, T., Zhu, L., Shiratori, Y., Ohte, N., Koba, K., Otsuka, S., Senoo, K., 2015. Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. The ISME Journal 9, 1954–1965.
Yu, K., Chen, G., 2009. Nitrous oxide emissions from terrestrial plants: observations, mechanisms and implications. In: Sheldon, A.I., Barnhart, E.P. (Eds.), Nitrous Oxide Emissions Research Progress. Nova Science Publishers, New York, USA, pp. 85–104.
Zou, J., Huang, Y., Sun, W., Zheng, X., 2005. Contribution of plants to N_2O emissions in soil-winter wheat ecosystem: pot and field experiments. Plant and Soil 269, 205–211.