Denitrifying bacterial communities vary with soil N in canola fields

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Introduction

The annual production of canola has increased sharply in the past decade in Canada. Canola crops require large inputs of N fertilizer. Especially the release of high-yielding hybrid cultivars in recent years has raised the amounts of N fertilizer applied in canola fields (Cutforth *et al*., 2009), which increases the carbon footprint of canola products (Gan *et al*., 2012). Therefore, it is important to understand the influence of N fertilization on the process of denitrification in canola fields. The objective of this study was to determine the effect of varying soil N availability on the community of denitrifiers inhabiting the rhizosphere of canola in western Canada.

Materials & methods

Experiment design and sample collection

A field experiment was conducted on Research Farms located in three different pedoclimatic zones of the Canadian prairie: Swift Current (Brown soil), Scott (Dark Brown soil), and at Indian Head (Black soil), SK, Canada. At each location, three soil N levels, no-N (0 kg N ha^{-1}), medium (60 kg N ha^{-1}), and high (120 kg N ha^{-1}), were randomized in four complete blocks, for a total of 36 plots. Soils first received the recommended levels of P, K and S based on soil nutrient tests. Then, the N fertilizer treatments were applied, i.e. 0, 130 and 260 kg ha^{-1} of urea, to create the no-N, medium, and high N level treatments in Brown and Dark Brown Chernozem zones, and the rates of 0, 118 and 249 kg ha^{-1} of urea were applied at Black Chernozem zone. The hybrid canola cultivar 'InVigor L150' was seeded at the rate of 8.8, 8.1 and 6.3 kg ha^{-1} at Brown, Dark Brown and Black Chernozem zones, respectively, to establish plant stands with a density of 150 plant m^{-2}. Rhizosphere soil samples were taken at the 50% flowering stage of canola from each plot. The rhizosphere soil was homogenized by sieving through 2-mm and placed in small plastic bags and stored at -20°C for molecular analysis.

Soil DNA extraction, PCR amplification and amplicons sequencing

Metagenomic DNA was extracted from the rhizosphere soils using the UltraClean Soil DNA Isolation Kit following the manufacturer's protocol. The extracted DNA was diluted 10-fold and subjected to polymerase chain reaction (PCR) with different specific designed primer sets targeting DNA fragments of the nitrite reductase genes *nirS* (cd3aF/R3cd), *nirK* (FlaCu/R3Cu) and the nitrous oxide reductase gene *nosZ* (nosZ-F/nosZ1622R). Fusion primers were prepared for each specific primer sets by adding sequencing adaptor A to the 5' end of forward primers, B adaptor to reverse primers, and multiplex identifiers (MIDs) between the A adaptor and the forward primers, in preparation for the production of barcoded amplicons libraries for each of the target genes. All sequence data received from Genome

Quebec were edited to remove primer, MID, and adaptor sequences using Mothur V.1.15.0. All data was analyzed by using R version 2.14.1.

Results

Increased soil N level influenced the structure of all three denitrifying communities differently (**Fig. 1**). The relative abundance of both the *nirK* and *nirS* gene-carrying α-proteobacteria was affected by N application, but the communities of these two gene carriers responded differently. In particular, the relative abundance of the *nirS* gene-carrying denitrifiers consistently decreased with increasing soil N level (P = 0.0478), whereas the relative abundance of the *nirK* gene carriers was suppressed by the high N application treatment (P = 0.0371).

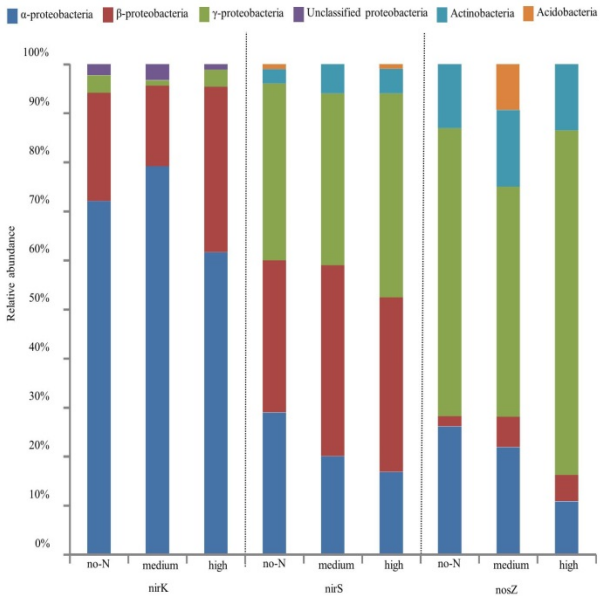

Fig. 1. The structure of the community of bacteria carrying the nitrite reductase genes nirK and nirS and the nitrous oxide reductase gene nosZ in canola rhizosphere soil (n = 12).

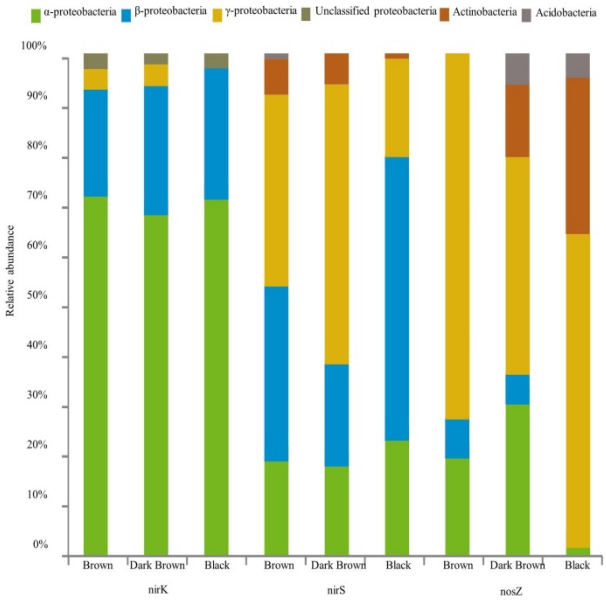

Fig. 2. Distribution of the two nitrite reductase genes *nirK* and *nirS* and of the nitrous oxide reductase gene *nosZ* among bacterial phyla, in the Brown, Dark Brown, and Black Chernozem soils (n = 12).

Soil N level influenced the relative abundance of the β-proteobacteria and α-proteobacteria carrying the gene *nirK* in contrasting ways (**Fig. 1**). The increase of soil N level significantly (P = 0.0500) increased the abundance of the *nirK* gene-carrying β-proteobacteria, but decreased the abundance of the *nirK* gene-carrying α-proteobacteria.

Pyrosequencing revealed the presence of denitrifying Acidobacteria (**Fig. 1**), and the relative abundance of the *nosZ* gene-carrying Acidobacteria increased with increasing soil N levels (P = 0.0327). The observation of the *nosZ* gene-carrying Acidobacteria only at the moderate N rate suggests that this group of bacteria has a narrow niche with certain soil N content, and may contribute to denitrification activity only within a narrow range of soil N fertility levels.

Different denitrifying bacterial communities were found at different soil zones (**Fig. 2**). The γ-proteobacteria carrying either the *nirK* ($P = 0.0080$) or the *nirS* ($P < 0.0001$) gene, and the α-proteobacteria carrying the *nosZ* gene ($P = 0.0380$) were more abundant in the Brown and Dark Brown Chernozems than in the Black Chernozem. On the contrary, β-proteobacteria containing the *nirS* gene ($P < 0.0001$) and Actinobacteria containing the *nosZ* gene ($P = 0.0100$) were more abundant in the Black Chernozem than in the Brown or Dark Brown Chernozems.

Conclusions

We found that the application of N fertilizer increases the potential for denitrification in canola rhizosphere by increasing the abundance of the genes coding for nitrite reductase and nitrous oxide reductase.

Soil N fertility management modified the composition of the denitrifying communities by changing the relative abundance of the denitrifying bacteria of different phylum.

We found that a high level of soil N increased the abundance of nitrite reductase gene-carrying denitrifiers more than nitrous oxide reductase gene-carrying denitrifiers, , and consequently, this may increase the rate of transformation of NO_3- to N_2O more than that of N_2O to N_2 in high N fertilized soils, and increase the risk of N_2O emissions in canola fields.

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