Influence of denitrifiers abundance on N_2O emissions in long term tillage system under a rainfed legume crop

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Current studies about nitrous oxide (N_2O) emissions from legume crops have raised considerable doubt, observing a high variability between sites (0.03-7.09 kg N_2O–N ha^{-1} y^{-1}) [1]. This high variability has been associated to climate and soil conditions, legume species and soil management practices (e.g. conservation or conventional tillage). Conservation tillage (i.e. no tillage (NT) and minimum tillage (MT)) has spread during the last decades because promotes several positive effects (increase of soil organic content, reduction of soil erosion and enhancement of carbon (C) sequestration). However, these benefits could be partly counterbalanced by negative effects on the release of N_2O emissions. Among processes responsible for N_2O production and consumption in soils, denitrification plays an important role both in tilled and no-tilled cropping systems [2]. Recently, amplification of functional bacterial genes involved in denitrification is being used to examine denitrifiers abundance and evaluate their influence on N_2O emissions. *NirK* and *nirS* are functional genes encoding the cytochrome cd1 and copper nitrite reductase, which is the key enzyme regulating the denitrification process.

The effect of conservation tillage vs. conventional tillage (CT) on denitrifiers abundance linked to changes on denitrification activity remains unclear and the studies conducted are scarce. Therefore, the aim of this study was to investigate possible interactions between denitrification key genes abundance and activity changes, under three tillage systems (NT, MT and CT) in a legume crop.

The experiment was conducted under controlled laboratory conditions, where denitrifiers abundance and potential denitrification were measured in three different soils. Soil samples were collected from a field-experiment in which long-term tillage systems was established in 1994. The tillage practices were NT, MT and CT and a crop rotation (fallow-wheat-vetch-barley) was established in each system. For this study, soil samples (0-10 cm depth) were only collected during vetch growing period (March 2011). The activity of denitrifier community was measured as potential denitrification using the acetylene blockage technique [3]. Relative abundance of *nirK* and *nirS* genes was quantified in those soils by independent real-time PCR assays.

The results showed that the products of potential denitrification (N_2O+N_2 and N_2) were affected by tillage practices. The N_2O+N_2 production was significantly higher ($P < 0.05$) in MT (8.01mg N $kg^{-1}d^{-1}$) than in NT (5.94 mg N $kg^{-1}d^{-1}$) and CT (4.82 mg N $kg^{-1}d^{-1}$). However, N_2 production rate (calculated as the difference between the N_2O produced in the presence and the absence of acetylene) was higher in NT (3.07 mg N $kg^{-1}d^{-1}$) than MT (2.26 mg N $kg^{-1}d^{-1}$) and CT (0.65 mg N $kg^{-1}d^{-1}$), but with no significant differences. The *nirK* copy numbers were higher than *nirS* copy numbers in all tillage systems. The *nirK* abundance in soil followed this trend NT > MT > CT but differences were not significant ($P > 0.05$). However, copy numbers of *nirS* gene were significantly higher ($P < 0.05$) for MT than NT and CT. Measurement of soil dissolved organic content (DOC) followed the order NT > MT > CT.

In conclusion, NT enhanced denitrification activity probably due to a higher DOC content compared to CT. Additionally, NT showed higher *nirK* and *nirS* gene abundances than CT, which would promote denitrification rates when soil conditions activated microbes in which these genes are present. A striking finding was that MT maintained a significant highest N_2O+N_2 production ($P < 0.05$) compared with NT and CT. This effect could be due to high DOC content together with the highest *nirS* abundance measured in MT.

Keywords: tillage; *nirK*; *nirS*; denitrifiers abundance; potential denitrification

References

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