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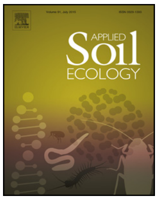

Over-winter dynamics of soil bacterial denitrifiers and nitrite ammonifiers influenced by crop residues with different carbon to nitrogen ratios

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ABSTRACT

The soil application of plant residues characterized by different carbon to nitrogen (C:N) ratios may lead to conditions more suitable to denitrifying and nitrite ammonifying bacteria. While the former microbial group fosters the loss of nitrogen (N) through the production of N_2 and nitrous oxide (N_2O), the latter may retain N into the agroecosystems. At present time there is no evidence on the effect of plant residues application on ammonifying bacterial populations over the winter. Dynamics of denitrifiers and nitrite ammonifiers abundance, composition and gaseous emissions (i.e. N_2O and CO_2) in soils following fall plough-down of barley (BRL) or red clover (RC) were evaluated at different time points for two consecutive winters in a humid temperate environment. Abundance of denitrifiers (*nirK* and *nirS*) and nitrite ammonifiers (*nrfA*) was greater in BRL-treated plots compared with RC, despite the higher C:N ratio of BRL. The present (DNA) and active (RNA) community structure of both denitrifiers and ammonifiers was different between BRL- and RC-treated plots and changed continuously during the two winters. The results suggested that both cold-induced edaphic conditions and crop residue application influenced and shaped the targeted functional communities. N_2O and CO_2 emission rates did not respond to crop residue source, however the emissions were 5–8-times greater in coldest months (i.e. January/February) compared to other dates during both winters. Our findings showed that cold-adapted denitrifying and nitrite ammonifying bacteria had a very similar response to the crop residues in abundance and diversity, suggesting that the application of contrasting C:N ratio crop residues did not create different niches for the nitrite ammonifiers and denitrifiers during the winter.

1. Introduction

Denitrification and dissimilatory nitrate reduction to ammonia (DNRA) are two soil processes favored under anoxic and micro-aerophilic conditions that can lead to the production of nitrous oxide (N_2O) gas (Rütting et al., 2011), a potent contributor to global warming and stratospheric ozone layer destruction (Hofstra and Bouwman, 2005). The denitrification pathway entails the stepwise reduction of NO_3^- to dinitrogen (N_2), and it is mainly performed by a wide range of heterotrophic bacteria (Zumft, 1997). Gaseous emissions of N_2O, an obligatory intermediate in the denitrification pathway, often occurs in agricultural systems. DNRA consists of the reduction of NO_3^- to ammonium (NH_4^+), mostly by heterotrophic and chemiolithotrophic bacteria named nitrite ammonifiers (Tiedje, 1988). Unlike denitrification, DNRA preserve nitrogen (N) in the soil system. Moreover, the final product (NH_4^+) is less susceptible to loss by leaching than the substrate (NO_3^-). However, nitrite ammonifiers are also capable of producing N_2O (Behrendt et al., 2015; Yoon et al., 2015), possibly to cope with toxicity associated with high NO_2^- concentrations (Kaspar, 1982). It has been shown that up to 10% of available NO_3^- can be converted to N_2O by nitrite ammonifiers in pure culture (Smith and Zimmerman, 1981). In soils, N transformation by

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denitrification and DNRA can be influenced by several factors, including NO_3^- and organic carbon (C) concentrations, soil redox potential, pH and temperature (Rütting et al., 2011). Previous studies have shown that denitrification may be favored by high soil NO_3^- concentrations (Tiedje et al., 1982), whereas DNRA may become the dominant process when NO_3^- concentration is limiting and organic carbon is available (i.e., high available C:NO_3^- ratio; Silver et al., 2005).

Nitrite reductase enzymes are involved in both denitrification and DNRA processes. In denitrification, the nitrite reductase is encoded by *nirS*, or *nirK* (Zumft, 1997), while in DNRA, NO_2^- is reduced to NH_4^+ by the NrfA enzyme encoded by the *nrfA* gene (Klotz et al., 2008). These functional genes can be used to evaluate the abundance and structure of denitrifiers and nitrite ammonifiers to determine the potential for nitrate reduction activity in the soil environment (Morales et al., 2010; Wallenstein et al., 2006).

Crop rotation and the resulting incorporation of residues in the soil are common agricultural practices used to increase aggregate stability, total C and N retention and crop productivity (Havlin et al., 1990; Jackson et al., 1993; Kumar and Goh, 1999; Guertal et al., 1997). In the growing season, it has been shown that the application of high C:N ratio crop residues, including barley and wheat straw, had no stimulating effect on N_2O emissions as a result of net N immobilization and biomass recalcitrance (Malhi and Lemke, 2007; Mutegi et al., 2010). However, greater N_2O fluxes were measured following the incorporation of crop residue with a low C:N ratio (using sugar beet and onion leaf tissue), due to high N release through mineralization (Kaiser et al., 1998; Toma and Hatano, 2007). When the effects of crop residue incorporation on denitrifiers were analyzed, an increase of their abundance was observed, both in microcosms and under field conditions compared to no residue treatments (Henderson et al., 2010; Miller et al., 2012; Sun et al., 2015). No investigation has been conducted so far to evaluate the effect of crop residues on soil nitrite ammonifying communities.

While extensive efforts have been made to study soil N cycling during the growing season, less information is available over the winter. N cycling is still active during the winter, and over 50% of the total annual N_2O emissions from agro-ecosystems can be released during the winter months (Wagner-Riddle et al., 1997; Virkajärvi et al., 2010). In higher latitudes, winter generally favors denitrification due to soil freezing that leads to a decrease in reduced soil gas exchange and hence reduced O_2 concentration, and disruption of soil aggregates and microbial cells resulting in increasing nutrient availability (Matzner and Borken, 2008; de Bruijn et al., 2009). To our knowledge, only one study has analyzed the impact of winter conditions on denitrifier abundance and related-N_2O emissions following crop residue incorporation in the field (Németh et al., 2014) and no study has compared the effect of crop residue on denitrifier and nitrite ammonifier community structure in agroecosystems over-winter.

The aim of this work was to determine the effect of incorporation of crop residues with contrasting C:N ratios (i.e., barley straw and red clover) on abundance and diversity of denitrifiers and nitrite ammonifiers, and on rates of N_2O emissions and denitrification, over-winter. We hypothesized that the contrasting C:N ratio of applied crop residues could selectively modulate the soil abundance and structure of either denitrifiers or the nitrite ammonifiers and influence the amplitude of over-winter N_2O emissions. The use of red clover (characterized by low C:N ratio) may offer greater availability of NO_3^- and readily utilizable carbon, therefore stimulating mineralization and subsequent depletion of O_2, creating conditions that are favorable to denitrifiers and conducive to denitrification. In contrast, the soil application of barley (characterized by high C:N ratio tissue) may result in limited NO_3^- availability and low mineralization of carbon, providing conditions that may be more suitable for nitrite ammonifiers and for DNRA to occur.

2. Materials and methods

2.1. Experimental site, cropping system and agricultural management

The field experiments were conducted at the Fredericton Research and Development Centre in Fredericton, New Brunswick, Canada (45°52′N, 66°108 31′W) during two winters (2009–2010 and 2010–2011). Soils at the experimental site belong to the Research Station (coarse loamy morainal ablational till over coarse loamy morainal lodgement till), and are classified as Orthic Humo-Ferric Podzols (FAO classification). Soil properties for the 0–15 cm depth were pH (1:1 water) of 6.2 and soil organic C and total N concentrations (LECO CNS–1000) of 19.2 g C kg^{-1} and 1.67 g N kg^{-1}, respectively for plots used in summer 2009. Soil textural class (pipette method with organic matter removal) was loam with 490 g kg^{-1} sand, 390 g kg^{-1} silt and 110 g kg^{-1} clay. The soil C:N ratio was 11.5. Although different plots were used for 2009–2010 and 2010–2011, soil properties were similar.

This study used a subset of treatments from an experiment established in 2007 to compare two-year potato cropping systems (Zebarth et al., 2012; Snowdon et al., 2013). For the purposes of this study, the experiment used a randomized complete block design with two crop rotation treatments replicated four times where plots were 5.5 m × 10 m in size. The treatments included potatoes grown with rotation crops of barley (*Hordeum vulgare* L.) or red clover (*Trifolium pratense* L.), chosen to be crop species which provide low and high C:N crop residues, respectively. Experimental plots were studied during the 2009–2010 and 2010–2011 over-winter periods, following rotation crops grown in the 2009 and 2010 growing seasons. Immediately before the crops were planted, all plots received a blanket application of 80 kg ha^{-1} of P_2O_5 and K_2O that was surface broadcast and incorporated with harrows in both years. Red clover and barley were machine planted on May 13 and 14, 2009, respectively, and on May 18· 2010. Barley cultivar Encore was seeded at 140 kg ha^{-1} using a grain drill. Nitrogen was applied at 67 kg ha^{-1} urea as a pre-plant broadcast and incorporated with harrows prior to seeding. Red clover cultivar AC Endure was seeded at 11 kg ha^{-1} with inoculant using a grain drill. No N fertilizer was applied. Barley was harvested on August 20, 2009 and August 17, 2010. The grain was harvested by combine and the straw was chopped and returned to the field. Red clover was chopped by flail mower and the residues were left on the field on October 2, 2009 and August 18, 2010. Barley and red clover plots were plowed on the same day on October 23, 2009 and November 12, 2010. Above-ground biomasses were measured in the fall of 2009. BRL straw contained approximately 1.09 t C ha^{-1} and 19 kg N ha^{-1} (based on harvest in August) while above-ground red clover tissue contained approximately 0.44 t C ha^{-1} and 16 kg N ha^{-1} (based on single harvest in October) in organic C and N, respectively. Total N applied over the growing season by both fertilizer application and plant residues was roughly estimated to be 38 kg N ha^{-1} and 16 kg N ha^{-1}, for BRL and RC, respectively. Red clover and barley straw had C:N ratios of 16 and 57, respectively.

Mean air temperature and total precipitation in the over-winter period (November to April) in Fredericton (NB) were −3.3 °C and 91.45 mm, respectively, between 1981 and 2009. Mean snow cover (1981–2009) was 12.6 cm (http://climate.weather.gc.ca).

2.2. Soil sampling and analysis

Soil samples were taken on five times during the non-growing season which is winter in Canada: November (after end of cropping