Applied Soil Ecology 77 (2014) 68–71

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Short communication

Bacteria dominate ammonia oxidation in soils used for outdoor cattle overwintering

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ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 24 January 2014
Accepted 27 January 2014
Available online 27 February 2014

Keywords:
Ammonia oxidation
Bacteria
Archaea
amoA diversity
Urea
Pasture

ABSTRACT

In areas used for cattle overwintering detrimental effects normally associated with grazing are intensified. Among the alterations observed, increases on the N availability and soil pH may highly influence structure of ammonia oxidizing microbes and thus influence nitrification pattern in soil. To evaluate this assumption, we assessed the abundance and diversity of ammonia oxidizing bacteria (AOB) and archaea (AOA) in three sites with different degrees of animal impact (severe, moderate or no impact) of an overwintering pasture by means of qPCR and T-RFLP of *amoA* genes. In areas where no animal impact could be identified AOA was dominating over AOB. However, AOB abundance increased as the degree of animal impact enhances, becoming most dominant in the severely impacted site. Interestingly, the diversity of AOB was the highest in the severely impacted area, where AOA diversity was the lowest. Obviously the pressure imposed by altered environmental conditions created by cattle husbandry lead to the selection of AOB and AOA populations, adapted to alkaline pH and higher ammonia concentration.

1. Introduction

Many grassland ecosystems are deeply influenced by animal grazing. These effects depend on many factors such as forage, soil physical properties, land use intensity and management (Wells and Doughtherty, 1997). Soil compaction is one of the most detrimental effects of grazing cattle on soils. It occurs mainly at the surface and might decrease soil permeability to air and water and limit root elongation (Mulholland and Fullen, 1991). Furthermore, the release of animal excrements increases nutrient availability in the soil. For instance, approximately 75% of the nitrogen ingested by cattle is released into soil, mainly in the form of urea. Once in soil, urea is rapidly transformed to ammonia (Petersen et al., 1998), which is partly lost via volatilization or used by most heterotrophic organisms to build up biomass respectively by ammonia oxidizing microbes as electron donor.

The process of ammonia oxidation (AO) is the first step of nitrification, in which ammonia (NH_3) is oxidized to nitrate (NO_3^-) via hydroxylamine (NH_2OH) and nitrite (NO_2^-). This is a key process of N cycling in the soils and determines to a large degree fertility pattern, as the formed NO_3^- may readily leach to deeper soil layers or used as alternative electron acceptor under oxygen limiting conditions. Even though recent data indicate a dominance of ammonia oxidizing archaea (AOA) compared to its bacterial counterpart (AOB) in pasture soils (Di et al., 2010; Taylor et al., 2010), the actual contribution of archaea to ammonia oxidation is still under debate. Mainly high loads of ammonia have been postulated to inhibit activity of AOA (Martens-Habbena et al., 2009). Thus it is an open question, if these grazing may change abundance patter of AOA and AOB in grassland soils.

Therefore, in the present study we investigated the effects of cattle husbandry on the diversity and abundance of AOA and AOB from an overwintering area, using the ammonia monooxygenase gene (*amoA*) as a functional marker. Highly impacted areas, where nutrient availability and soil physicochemical characteristics are significantly altered (Hynšt et al., 2007a), were compared to areas with no or only moderate cattle impact. We postulated that the increase of anaerobic micro-sites in the highly impacted areas of the pasture (Elhottová et al., 2012) would cause an decrease on the abundance of ammonia oxidizing microorganisms in general. As ammonia loads increase with the degree of animal impact, changes on the AOA/AOB ratio are expected, leading to a dominance of AOB.

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http://dx.doi.org/10.1016/j.apsoil.2014.01.008

2. Materials and methods

2.1. Experimental area

An area close to Český Krumlov in South Bohemia, Czech Republic (latitude 48°52′ N, longitude 14°13′ E) was chosen for this investigation, where more than 90 animals were kept on an area of approximately 4 ha since 1994/1995 (Hynšt et al., 2007b). The soil on the site is a sandy loam classified as Haplic Phaeozem (arenic; WRB system), containing 60–80% sand, 14–32% silt, and 6–14% clay. Relevant soil characteristics are described in Table 1 (Chroňáková et al., 2009). The presence of the animals during the period of October/November until April/May clearly affected the area, leading to changes on vegetation and soil characteristics (for more details see (Hynšt et al., 2007a,b; Šimek et al., 2006)). A gradient of animal impact from the most impacted areas near the animal barn (severely impacted, SI) to much less impacted areas in the middle (moderately impacted, MI) and almost unaffected areas at the opposite side of the overwintering area (no impact, NI) became apparent. At each of the three sites (SI, MI and NI) 4 replicate samples were taken from the topsoil (0–20 cm) in an area of 1 m^2, in spring (May) right after the overwintering season and in fall (October) before the animals came back to the area. The soil samples were immediately sieved through a 5 mm mesh sieve and stored at −80 °C for molecular biology analysis.

2.2. Real time PCR (qPCR) and terminal restriction fragment analysis (T-RFLP) of amoA genes

DNA was extracted from 500 mg of soil according to Griffiths et al. (2000). The quantity and quality of the extracts were evaluated using 1% agarose gels and spectrophotometer (NanoDrop Technologies, USA). In pre-experiments dilutions of the DNA extracts were determined that did not inhibit PCR reactions (data not shown). qPCR of the *amoA* gene for AOA and AOB was performed as described by Schauss et al. (2009). Efficiency values for AOA and AOB reactions were in 90.3% and 103.7%, respectively. The same primers as well as PCR- and cycler conditions were used for T-RFLP analysis of AOA and AOB *amoA* genes, except that the forward primers were FAM-labeled. PCR products were purified using QIAquick PCR purification Kit (Qiagen, Germany). Double restriction digestions tested in silico were designed to obtain a better resolution of the diversity. Following manufacturer's instructions, 20 U of BstUI and 10 U of TaqI (New England Biolabs, Germany) or 5 U FspBI and 10 U PsyI (Fermentas, Germany) were used to digest 20 μl of PCR product for AOB and AOA, respectively. Reaction products were purified with MinElute PCR purification kit (Qiagen, Germany) and analyzed in an ABI 3730 DNA Analyzer (Applied Biosystems, USA). Fragments with sizes smaller than 50 bp were excluded to avoid the detection of primer dimer peaks. In order to discriminate peak signal from background noise, data was standardized by dividing single peak areas by the sum of the total peak area of the particular sample. A peak was considered as true signal when it represented more than 1% of the total peak area.

2.3. Statistical analysis

The data matrices were exported for analysis within the R software environment (www.R-project.org). To analyze the relation between the AOA respectively AOB community structure and the factors site and season a between group analysis based on principal component analysis was carried out (function bga in R-package made4). To link the soil factors, characterized as group medians, with BGA scores spearman correlation coefficients were calculated. Additional specific methods for statistical analysis of presence/absence data were applied: biclustering (function biclust in R-package biclust) and prediction analysis of categorical data (function pamCat in R-package scrime).

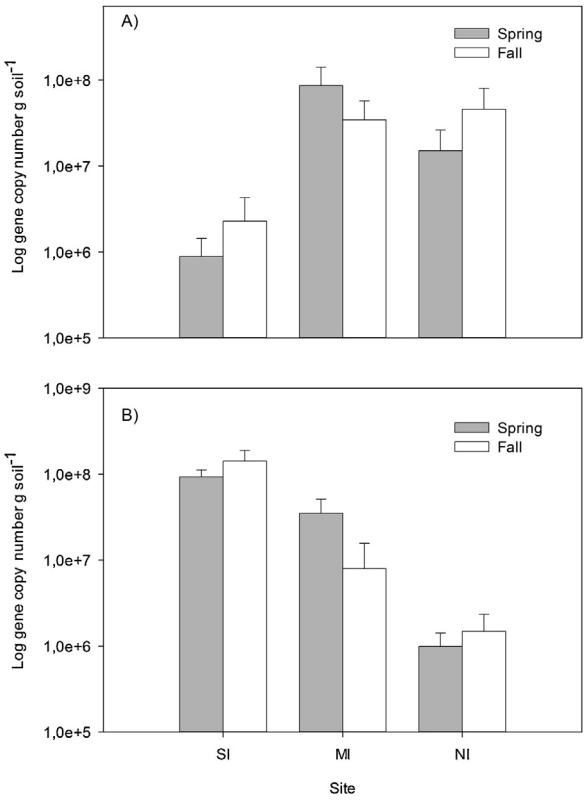

Fig. 1. (A) Abundance of archaeal (AOA) and (B) bacterial *amoA* genes (AOB) quantified in Borová soils in samples collected from severely impacted (SI), moderately impacted (MI) and non-impacted areas along the overwintering pasture area. Samples were collected in spring (May) right after the overwintering season and in fall (October) before the animals came back to the area.

3. Results and discussion

AOA dominated in soil samples from sites that were not impacted by cattle (NI). Gene copy numbers for archaeal *amoA* reached up to 1.3×10^7 copies g^{-1} soil dw and did not significantly change during spring and autumn sampling (Fig. 1). For AOB, *amoA* copy numbers were more than one magnitude lower, resulting in AOA:AOB ratios of 15.25 and 30.87 during spring and fall, respectively. At this site the lowest pH, organic carbon and ammonia concentrations were measured (Table 1).

The abundance of AOA and AOB was higher in soil samples obtained from MI and reached values of 8.6×10^7 and 3.5×10^7 copies g^{-1} soil for AOA and AOB, respectively (Fig. 1). Overall the ratio between AOA and AOB was significantly lower compared to NI and ranged about 2. The assumption that this finding would be related to ammonia availability could not be proven. Even though ammonium concentrations were 4 times higher in MI than NI, this difference was not statistically significant. The patchy distribution on ammonium, originated from animal urine, lead to great variation among the replicates, mainly in spring. Nevertheless, both, higher pH and hotspots with high amounts of ammonia might have favored AOB here. As shown by Di et al. (2009), AOB growth was stimulated by the application of urine to pasture soils. They also observed a significant decrease of the nitrification activity when dicyandiamide (DCD) was added. As DCD inhibits the growth of AOB but not AOA, the authors postulated that nitrification was driven by AOB rather than AOA in the investigated nitrogen-rich pasture soils.