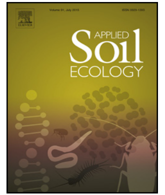
Applied Soil Ecology 114 (2017) 45–51

Contents lists available at ScienceDirect

Applied Soil Ecology

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



Divergent response of ammonia oxidizers to various amino acids

Vasileios A. Tzanakakis[a,*], Nikolaos V. Paranychianakis[b]

[a] Region of Crete, Directorate of Agricultural Economy, Iraklion 71201, Greece
[b] School of Environmental Engineering, Technical University of Crete, Polytechnioupolis, 73100 Chania, Greece

ARTICLE INFO

Article history:
Received 11 June 2016
Received in revised form 6 February 2017
Accepted 20 February 2017
Available online xxx

Keywords:
Nitrification
Amino acids
Mineralization
Ammonia oxidizing bacteria
Ammonia oxidizing archaea
DCD

ABSTRACT

Amino acids (AAs) have an important role on the biogeochemical cycle of N in terrestrial environments. However, the effect of AAs on chemolithotrophic ammonia-oxidizing bacteria (AOB) and archaea (AOA) and nitrification rate has not been elucidated. Supplying soil microcosms with three AAs (asparagine, glutamate, and phenylalanine) with distinct intrinsic properties and equivalent amount of ammonium (NH_4^+), we investigated their effect on AOB and AOB *amo*A gene abundances and on nitrification rate. A parallel experiment was also performed in which nitrification was inhibited, using dicyandiamide (DCD), to get insights on *amo*A-dependent nitrification activity and the mineralization of AAs. The source of N strongly affected nitrification with the highest rate to be observed in NH_4^+-treated soils followed by asparagine, glutamate, and phenylalanine. Nitrate accumulation was associated with an increase in the abundance of AOB *amo*A genes suggesting a stronger contribution of AOB in the regulation of nitrification compared to AOA. Differences in N-mineralization rate of AAs appear as the main driver of the divergent nitrification rates is soils treated with various sources of N, which probably was driven by their different properties. Moreover in the case of phenylalanine, inhibitory effects on nitrification were documented. Nitrification was completely inhibited by DCD, indicating *amo*A-dependent nitrification activity. The present study demonstrates the crucial role of AAs on performance of ammonia oxidizers and nitrification, particularly in neutral or slightly alkaline soils with low organic matter content.
© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Nitrification is a key process in the global biochemical nitrogen cycle comprising the biological oxidation of ammonia (NH_3), via nitrite (NO_2^-), to nitrate (NO_3^-). This two-step process is performed either by phylogenetically distinct groups of ammonia oxidizing bacteria (AOB) or archaea (AOA) and nitrite oxidizing bacteria, consecutively, or by members of *Nitrospira* genus capable of performing complete nitrification (Commamox bacteria) (Daims et al., 2015; van Kessel et al., 2015). Ammonia oxidation, beside its role to ecosystem productivity as a part of nitrification, is also a nitrous oxide (N_2O) yielding process contributing substantially to global N_2O emissions. Nitrous oxide can be produced either via nitrification-coupled denitrification process or directly via ammonia oxidation and/or nitrifier denitrification in atmospheric or hypoxic conditions (Wrage et al., 2001; Kool et al., 2011; Zhu et al., 2013). Nitrous oxide gas is a potent greenhouse gas with 298 times higher global warming potential than carbon dioxide over a100-year period, and responsible, also, for ozone depletion in the stratosphere (Ravishankara et al., 2009). Given the importance of ammonia oxidation in the terrestrial (and global) N cycling it is imperative to understand the ecology and physiology of ammonia oxidizers in order to develop the necessary strategies increasing ecosystems N use efficiency and reducing N losses to the environment (van Groenigen et al., 2014).

Ammonia oxidizers (AOB and AOA) coexist in a wide range of environments (Leininger et al., 2006) but their ecological preferences and their role in ammonia oxidation has not been yet elucidated. Previous studies have provided evidence that NH_3 availability is a dominant factor of the relative contribution of the two groups in ammonia oxidation (Prosser and Nicol, 2012; Taylor et al., 2012; Zhang et al., 2012; Lu et al., 2015; Taylor et al., 2015; Wang et al., 2015). Overall, AOA outweight AOB in oligotrophic environments, due to higher affinity of AOA for ammonia oxidation (Martens-Habbena et al., 2009) and/or to their greater sensitivity to NH_3 availability (Prosser and Nicol, 2012). By contrast, AOB drive nitrification in NH_3 rich environments (Di et al ., 2010). However, some reports, in which high levels of ammonium (NH_4^+) stimulated the abundance of AOA in absense (Verhamme et al., 2011) or presence of AOB inhibitors (Lu et al., 2015; Hink et al.,

* Corresponding author.
E-mail address: vetzanakakis@gmail.com (V.A. Tzanakakis).

http://dx.doi.org/10.1016/j.apsoil.2017.02.019
0929-1393/© 2017 Elsevier B.V. All rights reserved.

2016), indicate potential adaptation of AOA communities. Mixotrophy has been suggested as another physiological characteristic that might cause differentiation in AOB and AOA niches (Mussmann et al., 2011; Prosser and Nicol, 2012; Lehtovirta-Morley et al., 2014), an assumption compatible with the distinct physiological characteristics between AOB and AOA (Hatzenpichler, 2012; Zhalnina et al., 2012). AOA are able to assimilate low molecular-weight organic compounds, as has been demonstrated experimentally in an uncultivated marine crenarchaeote (*Cenarchaeum symbiosum*) (Hallam et al., 2006) and in an isolated soil AOA the *Nitrososphaera viennensis* (Tourna et al., 2011). Evidence for a mixotrophic lifestyle of AOA has been provided by microcosm (Jia and Conrad, 2009; Levicnik-Hofferle et al., 2012; Paranychianakis et al., 2013) and field (Chen et al., 2008; Wessén et al., 2010) studies. However, for the DDS1 strain of AOA, although it was able to uptake α-keto acids (e.g., pyruvate, oxaloacetate), they were used for the scavenging of H_2O_2 (Kim et al ., 2016).

Amino acids (AAs), are an important source of C and N in terrestrial environments, which could affect the population and activity of ammonia oxidizers. This can be attributed to different C and N availability, controlled by the intrinsic AA characteristics and the interactions with soil matrix and heterotrophic microorganisms (Jones and Hodge, 1999; McLain and Martens, 2005; Geisseler et al., 2010). In addition, direct effects of AAs on ammonia oxidizers driven by AA-specific requirements by ammonia oxidizers and/or inhibition phenomena have been reported (Clark and Schmidt, 1967; Gundersen, 1955). Earlier work indicates that the C:N ratio (Geisseler et al., 2009), charge (Jones and Hodge, 1999; Rothstein, 2010) and polarity/hydrophobicity of AAs (McLain and Martens, 2005; Rothstein, 2010) as important properties that eventually determine their fate in the soil and hence on the N availability (Geisseler et al., 2010). The C:N ratio of AAs is a critical property affecting the mineralization of AAs (Geisseler et al ., 2009). Polarity of AAs has been associated with increased mineralization potential (Rothstein, 2010) whereas positively charged AAs are preferentially sorbed onto soil particles (Jones and Hodge, 1999; Rothstein, 2010).

This study aims to investigate the effect of specific AAs (asparagine, glutamate, and phenylalanine) with different intrinsic properties on autotrophic ammonia oxidation. To achieve the objectives repeated additions of AAs and NH_4^+ were applied in microcosms and the transformation of N and the abundance of *amo*A gene of AOB/AOA were monitored over 32-day period. The nitrification inhibitor dicyandiamide (DCD) was also used to estimate the mineralization rate of AAs as a driver of NH_3 availability and to examine the *amo*A dependence of ammonia oxidation driven by AOB or AOA.

2. Material and methods

2.1. Establishment of soil microcosms

The microcosm experiments included the following treatments: (i) controls that treated with deionized water, (ii) soils treated with ammonium sulfate, and (iii) microcosms treated with aminoacids (asparagine, glutamic acid, and phenylalanine) (Sigma–Aldrich). Fifteen replicates were included in each of the treatments. To prepare the soil microcosms, 15 g of soil, collected from the upper 20 cm of an olive orchard, were added to 50-ml falcons. The olive orchard (Crete-Greece) was subjected to inorganic fertilization on an annual basis (~150 kg N ha^{-1} yr^{-1}). The soil was classified as Calcisol (FAO-UNESCO, 1990), Clay-loam (CL), with pH: 7.56; NH_4^+-N: 37.81 mg kg^{-1} d.w.; and soil organic matter 1.50%. Before establishing microcosm experiments, soil samples were sieved (8 mm mesh size) and stored at 4 °C for a month. Soil gravimetric water content was determined using 15-g samples dried at 105 °C for 48 h.

The amino acids (asparagine, glutamic acid, and phenylalanine) were selected on basis of their different intrinsic properties (C/N ratio, hydrophobicity, and polarity) and potential effects on ammonia oxidizers (Levicnik-Hofferle et al., 2012). The experiment included four applications of 100 N μg g^{-1} for each nitrogenous compound every four days (0, 4, 8, and 12 day). The soil moisture content was maintained to 60% of water holding capacity (−90 kPa soil water potential) by adding deionized water. Parallel microcosms, including the same treatments, were prepared and treated with the nitrification inhibitor (DCD) added to the soil at the rate of 10 μg g^{-1} every fourth day.

The soil microcosms were incubated at 25 °C in darkness and triplicates from each treatment were destructively sampled at days 0, 8, 16, 24, and 32. Sub-samples from each microcosm were stored immediately at −20 °C for DNA extraction, while the remaining sub-sample was used for the determination of NH_4^+-N and NO_3^--N and pH. Soil NH_+^4-N and NO_3^--N contents were measured colorimetrically in a PerkinElmer Lambda 25 spectrophotometer with the Nessler and the Cd reduction method, respectively. The extraction of soil NH_4^+-N and NO_3^--N was performed with 2 M KCl and 0.01 M $CaCl_2$, respectively. The pH was determined in soil suspensions (1:5, soil/0.01 M $CaCl_2$) after the mixing and settling.

2.2. DNA extraction and quantitative PCR analyses

Genomic DNA was extracted from 0.25 g of soil, frozen beforehand and homogenized with a mortar, using the Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc. Carlsbad, CA, USA). DNA quality was checked in 1% agarose gel, and quantified in a Lambda 25 spectrophotometer and stored at −80 °C. PCR amplification of AOB and AOA was carried out with the primer pairs amoA-1F/amoA-2R (Rotthauwe et al., 1997), and amoAF/amoAR (Francis et al., 2005), respectively. More detailed, 250 nM primer concentration was used while the cycling conditions included 3 min denaturation step at 95 °C, followed by 35 cycles of 30 s at 95 °C, 35 s at 57.5 °C (55 °C for AOA) and a data acquisition step at 84 and 81 °C, respectively for 15 s.

Quantification of gene copy numbers was performed with the StepOnePlus™ Real-Time PCR System (Applied Biosystems) in 20 μl reactions using the KAPA SYBR Fast Master Mix (2×) qRT-PCR Kit (KAPA Biosystems) and 1 μl of 1/20 diluted soil DNA. All reactions were completed with a melting curve starting at 60 °C with an increase of 0.5 °C up to 95 °C to verify amplicon specificity. Standard curves were obtained using serial dilutions, 10^3–10^8 for ammonia oxidizing organisms of linearized plasmids (pGEM-T, Promega) containing cloned *amo*A genes amplified from the soil of this study. Controls without templates resulted in undetectable signal in all the runnings, while no inhibitory effects were detected at the adopted dilution. The amplification efficiencies were 83% for AOB and 90% for AOA.

2.3. Statistical analysis

ANOVA combined with post hoc Tukey test was used to estimate for each sampling day the differences in NO_3^--N and NH_4^+-N content, soil pH, and AOB/AOA *amo*A abundances among the treatments. Net nitrification rate was determined using linear model taking into account the whole period of the study (32 days). Pearson correlation analysis was carried out between NO_3^--N and AOA/AOB *amo*A gene copy numbers. The values reported in this study are mean ± standard error of triplicate microcosms. All statistical analyses were done using SPSS 19.0 software.

Download English Version:

https://daneshyari.com/en/article/5742711

Download Persian Version:

https://daneshyari.com/article/5742711

Daneshyari.com