Contents lists available at ScienceDirect

Soil Biology and Biochemistry

Long-term effects of grazing, liming and nutrient fertilization on the nitrifying community of grassland soils

ARTICLE INFO

ABSTRACT

Human-managed grasslands receive significant inputs of fertilizing materials, which can greatly influence soil biological processes associated with the cycling of nitrogen (N), including microbial nitrification. Here we specifically address how soil ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) respond to 23 years of different management practices in a permanent grassland experiment.

We found that AOB *amoA* gene copy numbers were significantly higher in limed soils (associated with greater pH values) whereas AOA *amoA* gene copy numbers were higher in grazed grasslands. The chronic addition of inorganic N fertilizer in the form of ammonium nitrate (either applied alone or in combination with other macro-nutrients) greatly contributed to increase AOB abundance. Our study brings evidence of how soil-nitrifying communities can differently respond to the long-term effect of animal (i.e. rabbit) grazing and to repeated applications of nutrient (e.g. NH_4NO_3) and non-nutrient (i.e. $CaCO_3$) fertilizing materials.

Across soil ecosystems ammonia oxidation is the first critical step of the nitrification process, which plays an important role in ecosystem nitrogen (N) cycling. Nitrification is carried out by important microbial guilds such as ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) (Gruber and Galloway, 2008; Jia and Conrad, 2009; Di et al., 2010). The ammonia monooxygenase (AMO) gene carries out the first step of ammonia oxidation by catalysing the oxidation of ammonia (NH_3) to hydroxylamine (H_2NO), which is subsequently transformed to nitrite (NO_2^-) and then nitrate (NO_3^-) (Norton et al., 2002).

Nitrification can also be catalysed by 'complete ammonia oxidisers' ('comammox'), which have been recently identified within the *Nitrospira* genus (Hu and He, 2017). Significant comammox *amoA* gene copy numbers were recently found in waste water treatment plants and also in beech forest soils where they can exceed archaeal *amoA* gene copy numbers (Pjevac et al., 2017).

In this study, we primarily focus on AOA and AOB groups mainly because AOB *amoA* gene copies can be particularly abundant in human-managed soils (Long et al., 2012; Dai et al., 2013; Hill et al., 2015) and especially in soils receiving long-term inorganic N fertilizer additions (Zhou et al., 2015). Archaeal (AOA) *amoA* genes can also be numerically dominant compared to AOB across many soil ecosystems (Martens-Habbena et al., 2009; Gubry-Rangin et al., 2010; Onodera et al., 2010; Morimoto et al., 2011; Chen et al., 2014). Despite the numerical abundance of *amoA* genes, previous studies show that this does not necessarily equate to functional dominance, for example, AOA *amoA* gene numerical abundance was found in association with greater nitrification by AOB (i.e. functionally dominant AOB *amoA* genes) (Onodera et al., 2010; Morimoto et al., 2011).

Previous studies have shown how common management practices such as grazing, agricultural liming and nutrient fertilization can significantly alter soil-nitrifying communities (Di et al., 2010; Hayden et al., 2010; Dai et al., 2013; Xie et al., 2014; Hill et al., 2015). For example, liming applications and associated changes in soil pH can strongly increase AOB gene copy numbers but not AOA copy numbers (Yao et al., 2011). On the other hand, AOA can better respond to long-term organic N additions whereas AOB may benefit more from repeated inorganic fertilization (Zhou et al., 2015). Finally, AOB are better able to utilize ammonia (Prosser and Nicol, 2012), which is abundant in urine patches created by animal grazers. So far very few studies have, however, simultaneously addressed the long-term (> 20 years) potential effects of (a) liming, (b) grazing and (c) different combination of inorganic nutrient fertilizers on the relative abundance of AOA and AOB in permanent grassland soils.

This study aims to address how common management practices (i.e. grazing, liming and nutrient fertilization) might affect AOA and AOB abundance in a long-term grassland experiment established in Berkshire, UK in 1991. The grassland experiment is organized in a split plot design where (a) rabbit grazing (presence/absence), (b) liming (presence/absence) and (c) five different nutrient treatments (i.e. N-only, P-only, NPMg, NPKMg and Control) are nested within each other and included in each of 4 randomly located large blocks (see Supplementary Material for more details). Across the split-plot experiment we first collected five soil samples from each of 80 experimental plots (4 m^2 each) in June 2014 (0–20 cm soil depth) and then the soils were homogenised into a composite sample. Quantitative polymerase chain reaction (qPCR) was performed to quantify gene copies of archaeal and bacterial *amoA* genes (see Supplementary Material for more details). Our study set out to test the following hypotheses:
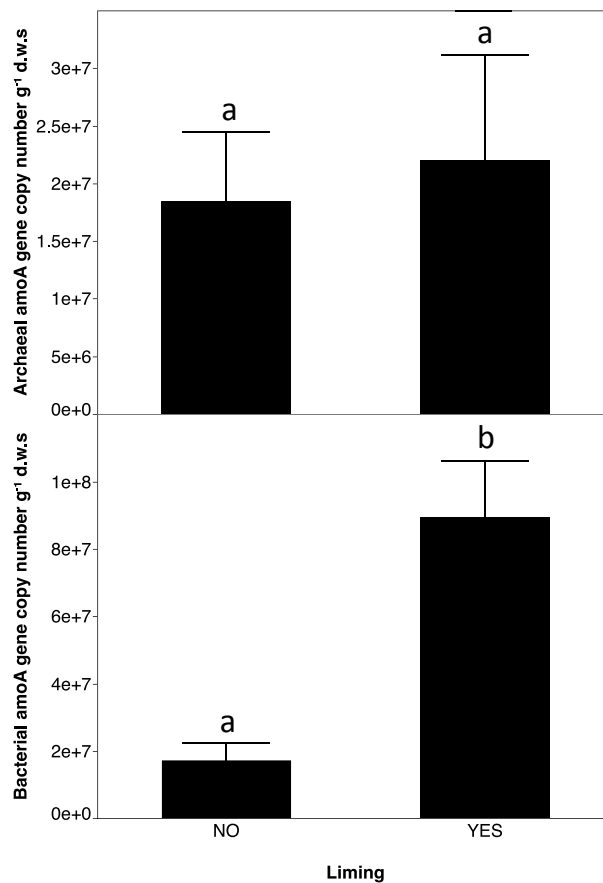
Fig. 1. Ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) abundance estimated as *amoA* gene copy numbers per gram of dry weight soil (g. d.w.s.) in response to liming applications. Error bars represent the standard error of mean values among plots receiving the same treatment. Significant differences between factor levels were tested using post-hoc Tukey tests. Different letters indicate a significant difference (P < .05).

H1. Liming will increase AOB *amoA* gene copy numbers but not AOA gene copies.

H2. AOA:AOB ratios will decrease under chronic rabbit grazing due to potential ammonia-inhibitory effects on AOA abundance.

H3. Nutrient treatments which include inorganic N additions will be associated with higher AOB gene copy numbers compared to other nutrient treatments, which do not include N.

We found that AOB copy numbers were on average ∼2.6 times greater than AOA. Average AOA copy number was $2.02 \times 10^7 \, g^{-1}$ d.w.s. whereas average AOB copy number was $5.32 \times 10^7 \, g^{-1}$ d.w.s. Ammonia oxidizing archaea *amoA* copy numbers ranged from 4.26×10^4 to $2.7 \times 10^8 \, g^{-1}$ d.w.s., whereas AOB *amoA* copy numbers ranged from 1.41×10^4 to $6.02 \times 10^8 \, g^{-1}$ d.w.s. Greater abundance of AOB compared with AOA could result from overall positive inorganic N fertilization effects on AOB *amoA* copy numbers (Zhou et al., 2015).

We found that the long-term addition of lime ($CaCO_3$) to soils increased AOB *amoA* gene copy numbers but had no significant effect on AOA *amoA* gene abundance ($F_{1,40} = 27.1$, P < .0001; Fig. 1), which confirms our first hypothesis (Nicol et al., 2008). Our evidence is that even after 23 years of $CaCO_3$ additions, these grassland soils remained slightly acid (mean soil pH is 4.57 in control plots and 6.58 in limed plots) thus being within the preferred soil pH range of AOB *Nitrosospira* clusters 2 and 3 that prefer acid and neutral soils respectively (Nicol et al., 2008). The large increase in AOB *amoA* gene copies within lime treated soils, therefore, maybe due to an increase in Nitrosospira cluster 3 where soils are closer to a neutral pH. The reduced abundance of AOB *amoA* gene copies in unlimed soils could be because many AOB have limited activity at pH < 6.5 (Koper et al., 2004; Nicol et al., 2008; Gubry-Rangin et al., 2011). Many studies have also found that AOB abundance correlates with pH whereas AOA are largely unaffected by this factor (Prosser and Nicol, 2012). No difference in AOA amoA gene copies between limed and unlimed soils could be because AOA groups adapt to acidic, acido-neutral or alkaline conditions (Gubry-Rangin et al., 2011).

Grazing had a positive effect on archaeal *amoA* ($F_{1,40} = 5.79$, P = .02; Fig. 2), which is contrary to our second hypothesis. Inhibition by ammonia, from urine, was hypothesised as a factor that would reduce AOA gene copies within grazed grassland plots. Increases in AOA under grazing treatment could be because of potential increases in humic acid inputs via faecal deposits from grazers, which may have cancelled out any inhibitory effect of ammonia on AOA abundance. It could also be that ammonium concentrations were too low in this grassland to impact this microbial functional guild.

We found that different combinations of inorganic nutrient amendments to soils affected AOA *amoA* gene copy numbers, which decreased in response to additions of NPKMg and N-only additions ($F_{4,40} = 3.89$, P = .009; Fig. 3). Ammonia oxidizing bacteria copy numbers were significantly