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Mineralization and nitrification: Archaea dominate ammonia-oxidising communities in grassland soils

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ABSTRACT

In grasslands, N mineralization and nitrification are important processes and are controlled by several factors, including the *in situ* microbial community composition. Nitrification involves ammonia oxidising archaea (AOA) and bacteria (AOB) and although AOA and AOB co-exist in soils, they respond differently to environmental characteristics and there is evidence of AOA/AOB niche differentiation. Here, we investigated temporal variation in N mineralization and nitrification rates, together with bacterial, archaeal and ammonia-oxidiser communities in grassland soils, on different geologies: clay, Greensand and Chalk. Across geologies, N mineralization and nitrification rates were slower in the autumn than the rest of the year. Turnover times for soil ammonium pools were <24 h, whilst several days for nitrate. In clay soils, bacterial, archaeal, AOA, and AOB communities were clearly distinct from those in Chalk and Greensand soils. Spatially and temporally, AOA were more abundant than AOB. Notably, *Nitrososphaera* were predominant, comprising 37.4% of archaeal communities, with the vast majority of AOA found in Chalk and Greensand soils. AOA abundance positively correlated with nitrate concentration, whereas AOB abundance correlated with ammonium and nitrite concentrations, suggesting that these N compounds may be potential drivers for AOA/AOB niche differentiation in these grassland soils.

1. Introduction

Grasslands cover ~40% of the Earth's land surface, are high in organic matter and represent a large reservoir of nitrogen (N) (Cambardella and Elliott, 1992). In the United Kingdom, grasslands cover over a fifth of the land area (ca. 7.5 million ha) and includes dry acid grasslands comprising 278,866 ha, calcareous Chalk grasslands covering 788,979 ha and improved grassland with over 3 million ha (Carey et al., 2007; Rodwell et al., 2007; Natural England, 2008). Grasslands were also identified as a priority habitat under the UK Biodiversity Action Plan (BAP) as they are important habitats for biodiversity and carbon sequestration; with UK grasslands sequestering 240 ± 200 kg of carbon per hectare per year (Natural England, 2008; Ostle et al., 2009).

Mineralization of soil organic matter by microorganisms is an important process in grassland N cycling, and is crucial for regulating the available N in soils for plant growth as well as preventing a net loss of N from the environment (Yao et al., 2011; Van der Heijden et al., 2008; Zhang et al., 2012). Previous work has shown that rising mean annual temperatures may increase net N mineralization in grassland soils, increasing coupled nitrification-denitrification, thus causing additional greenhouse gas contribution from grasslands, and a net loss of N from these ecosystems (Smith et al., 2002; Hutchinson, 1995; Zhang et al., 2012). Other factors may also control soil N mineralization and nitrification rates, including soil organic nitrogen (SON), soil water availability, total N, soil organic C (SOC), C:N ratio and microbial community composition (Dalal and Meyer, 1987; Fisk and Schmidt, 1995; Von Lutzow and Kögel-Knabner, 2009).

Ammonia oxidising bacteria (AOB) and archaea (AOA) are the major drivers of the aerobic oxidation of ammonia, which is particularly important for soil fertility (Könneke et al., 2005; Prosser and Nicol, 2008, 2012). Although AOA and AOB co-exist in soils, they respond differently to environmental factors and there is evidence of niche differentiation between AOB and AOA (Erguder et al., 2009; Prosser and Nicol, 2012; Hink et al., 2018). For example, the global dominance of AOA in acidic soils (Prosser and Nicol, 2008, 2012; Gubry-Rangin et al., 2011), and AOA, rather than AOB, favouring low ammonium

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environments, such as unfertilised soils (Leininger et al., 2006; Di et al., 2010; Gubry-Rangin et al., 2010; Verhamme et al., 2011; Hink et al., 2017). In grazed grasslands the abundance of AOA was found to be higher than that of AOB, and changes in AOA community composition correlate with changes in C:N ratio, whereas changes in AOB communities are independent of edaphic factors (Xie et al., 2014). Other studies however, showed that nitrate concentration correlated with AOB abundance (Di et al., 2009, 2010; Wertz et al., 2012). Furthermore, the recent discovery of the complete oxidation of ammonia to nitrate in a single organism (commamox) within the *Nitrospira* genus (Daims et al., 2015) and the co-occurrence with AOB has raised further questions on the niche specialization of nitrifiers in terrestrial ecosystems.

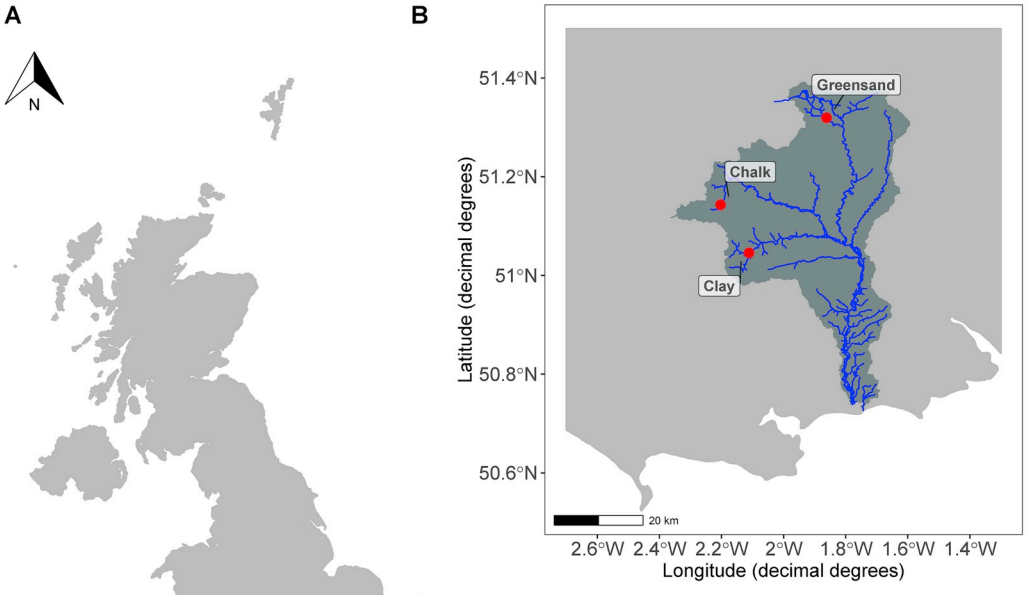
To determine the contribution of grasslands to global N budget, particularly under a changing climate, it is important to better understand grassland N dynamics and the associated microorganisms involved. In the UK, nitrate runoff from grasslands is one of the main sources of anthropogenically-enhanced nutrient loads across all river catchments (Nedwell et al., 2002; Earl et al., 2014). This is particularly important in permeable geologies where responses to land management changes targeted at reducing nitrate loadings are delayed due to long water residence times (Heppell et al., 2017). Given that England has more permeable Chalk rivers than any other country in Europe (around 3900 km) (Natural England, 2008), it is important to understand how changes in N dynamics across catchments potentially change nitrate production and delivery into rivers. Thus, examining grassland N dynamics, in relation to the microbial communities driving these processes, feeds directly into a landscape-scale understanding of N budgets. Here, we focussed on grassland soils, adjacent to rivers in southern United Kingdom (Fig. 1). The aims of this study were to investigate bacterial, archaeal and ammonia-oxidiser communities in grassland soils, on different geologies: clay, Greensand and Chalk. We hypothesized that the less permeable clay and more permeable Greensand and Chalk soils would select for different ammonia-oxidiser communities spatially and temporally, which in turn would drive changes in N mineralization and nitrification rates.

2. Methods and materials

2.1. Site descriptions and sampling

The research was undertaken at the Hampshire Avon catchment (southern England) which has sub-catchments of contrasting geology: clay, Greensand and Chalk (Allen et al., 2014; Heppell et al., 2017). The soils used in this study are herein referred to as clay (>99% Late Jurassic Kimmeridge Clay), Greensand (50% Upper Greensand) and Chalk (80% Chalk geology) soils based on their respective underlying geologies (Heppell et al., 2017) (Fig. 1). Soil descriptions are as follows: slowly permeable, clayey soil (clay); free-draining loamy soil (Greensand); permeable, base-rich loamy soil (Chalk soil) (Cranfield University, 2020). Over the sampling period, soil pH ranged from the more acidic clays pH 5.5, to 7.2 and 7.6 for Greensand and Chalk respectively. During the sampling period the catchment received a total of 824 mm rainfall (AEDA, accessed 2019).

To encompass any lateral gradients within and across sub-catchments, a random 100 × 100 m square was marked by stakes at right angles to the adjacent river at each location. Spatially independent and randomised replicate ($n = 8$) surface soils, were collected from each location (0–10 cm depth), in 2013–14: spring (April/May), summer (August), autumn (November), and winter (February/March) to cover the range of temperatures throughout the year (autumn/winter: 7 °C; spring/summer: 20 °C). These were typical temperatures for the region, consistent with temperatures we measured the previous year, which



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	Clay site	Greensand site	Chalk site
Underlying geology[a]	>99% Kimmeridge Clay	50% Upper Greensand	80% Chalk
Adjacent river[a]	Sem	Avon	Wylye
Major land use[a]	Grassland (90–95%), Arable (5–10%)	Grassland (50%), Arable (25%)	Grassland (35%), Arable (50%)
Catchment size[a]	4.9 km^2	59.2 km^2	53.5 km^2
Soil pH	4.9 ± 0.2	7.1 ± 0.3	7.6 ± 0.2

[a]Data modified from Heppell *et al*., 2017

Fig. 1. Map of sampling locations within the UK (A), and within the Hampshire-Avon catchment (B). The catchment area is shaded dark grey, whilst river channels are blue. Further geology and catchment details are given in (C).

ranged between 4 and 21 °C. Soil water content varied between summer lows of 26% in clay, 46% and 49% in Greensand and Chalk respectively; to autumn highs near 50% for clay and Greensand, and 80% for Chalk. Soil samples were maintained at *in situ* temperatures until processing (within 24 h). Sub-samples for molecular analyses were snap frozen on-site using a cryoshipper (−150 °C) and stored at −80 °C. Gravimetric water content of soils were determined by drying approximately 10 g of soil at 105 °C to constant weight, (expressed as g water g^{-1} of oven-dry soil).

2.2. Mineralization and nitrification rates

Both mineralization and nitrification rates were quantified using ^{15}N isotope microdiffusion techniques (Barraclough, 1991; Davidson et al., 1991; Myrold and Tiedje, 1986). In preliminary tests of NH_3 diffusion and ^{15}N recovery, two levels of ^{15}N labelling were used: natural abundance and a^{15}N enrichment of 9.2% of total NH_4^+. Ammonium chloride solution (50 ml of 100 μM NH_4Cl in 2M KCl) with known ^{15}N (%) enrichment was added to a serum bottle (100 ml). Half a microfibre filter (Whatman GF/C, 25 mm diameter) was suspended on a hook hanging beneath a rubber stopper and 50 μl of 2.5M $KHSO_4$ solution dropped onto the filter. The soil extract was made alkaline by adding 0.2 ml 2M NaOH and the bottle stirred for 10 min. Ammonium in the solution was converted to ammonia which diffused from solution to the headspace of the serum bottle and trapped on the acidified filter. The bottles were held at room temperature for 7 days until all the ammonia was absorbed onto the filter. The filter was removed, placed in a vial and dried in a desiccator. Recovery of NH_4^+ was >99%.

To measure mineralization, soil cores (~9 g wet weight: 0–10 cm depth) were taken in 10 ml plastic syringes with the distal end removed. Representative cores were extracted with 40 ml 2M KCl solution, shaken for 10 s and placed on a roller for 1 h. Tubes were centrifuged (4000 rpm, 10 min) (Sanyo, Harrier 15/80 MSE), the supernatant decanted and filtered (Whatman No 1 filter paper) and ammonium and nitrate concentrations in the soil extracts were analysed colorimetrically (Johnson and Coletti, 2002; Krom, 1980). The remaining cores were injected with 0.5 ml $(^{15}NH_4)_2SO_4$ (between 0.5 and 2.5 μg N g^{-1} dry soil for clay; between 1.5 and 3 μg N g^{-1} dry soil for Greensand; between 2.5 and 5.5 μg N g^{-1} dry soil for Chalk) to give 30% enrichment of $^{15}NH_4^+$. Duplicate samples were immediately extracted for time zero (t_0) controls to determine the extraction efficiency of the ^{15}N (which was >99%). The remaining soil samples were incubated for 24 h at *in situ* temperature, with their upper surfaces exposed to air.

Nitrification rates were measured by injecting further soil cores with 0.5 ml $K^{15}NO_3$ solution (between 10 and 20 μg N g^{-1} dry soil for clay; between 17 and 34 μg N g^{-1} dry soil for Greensand; between 20 and 35 μg N g^{-1} dry soil for Chalk) to give 20% enrichment of $^{15}NO_3$. Time zero (t_0) controls were immediately processed to determine the extraction efficiency of the ^{15}N (which was >97%) and the remaining samples incubated for 24 h at *in situ* temperatures. After incubation, samples were extracted with 2M KCl. The ammonia was then removed by making alkaline with 0.2 ml 2M NaOH solution and left for 7 days to absorb onto acidified filter paper (McMurray et al., 2002). Residual nitrate in the soil extract was then reduced to ammonia over 8 days using 0.2 g of MgO and 0.4 g of Devarda's alloy (Sigma- Aldrich). Ammonia from the reduction of NO_3^- was diffused out of solution over 8 days and was absorbed by the acidified filter. After 8 days, the filters were removed and dried in a desiccator. All $^{15}N/^{14}N$ isotopic measurements for samples were analysed by the NERC Life Sciences Mass Spectrometry Facility (LSMSF), Natural Environmental Research Council, UK (See Supplementary Information). Rates of mineralization, reassimilation, consumption and nitrification were calculated according to Kirkham and Bartholomew (1954).

2.3. Soil organic carbon (SOC) and soil organic nitrogen (SON)

Soil samples (0–10 cm depth) were taken with cut-off 10 ml sterile hypodermic syringes. Soils were sieved and ground in a ball mill, and samples for SOC were acidified with 0.5 ml 1% (v/v) HCl to remove carbonates from the soil before SOC analysis. Samples were oven dried at 105 °C to constant weight, cooled and sealed prior to analysis. Aliquots (20 mg) of samples were enclosed in tin sheets and placed in a pellet press to remove any air. SOC and SON were analysed by the Central Chemistry Unit of the NERC Centre for Ecology and Hydrology, Lancaster, UK. SOC was measured in a Vario EL (Elementar Analsensysteme GmbH, Hanau, Germany) (See Supplementary Information).

2.4. 16S rRNA and amoA gene analysis

DNA was extracted from 0.25 g wet weight soil using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc). Gene abundance was quantified by qPCR with a SensiFAST SYBR No-ROX Kit (Bioline) on a CFX96 Real-Time PCR Detection System (BioRad). Ammonia monooxygenase (*amoA*) genes were targeted with the primers amoA-1F and amoA-2R for AOB (Rotthauwe et al., 1997), and CrenamoA-23F and CrenamoA-616R for AOA (Tourna et al., 2008). Gene abundances were quantified with an absolute quantification method against an internal standard calibration curve using DNA standards of each target gene from 10^2 to 10^7 copies in 20 μl reactions containing 200 nM of primers and 1 μl of DNA template. Cycle conditions for all genes were 95 °C for 3 min followed by 40 cycles at 95 °C for 10s then 60 °C for 30 s. Amplification specificity was confirmed by melting curve analysis.

Amplicon libraries were prepared by a 28-cycle (16S rRNA Bacteria) or 31-cycle (16S rRNA Archaea, AOB and AOA *amoA* genes) PCR using the same locus-specific *amoA* gene primers as the qPCR assays and the variable regions 3–4 of the 16S rRNA genes were targeted using the primer pairs Bakt_341F (CCTACGGGNGGCWGCAG) and Bakt_805R (GACTACHVGGGTATCTAATCC) for Bacteria (Herlemann et al., 2011), and 344F (ACGGGGYGCAGCAGGCGCGA) (Raskin et al., 1994) and 915R (GTGCTCCCCCGCCAATTCCT) (Stahl and Amann, 1991) for Archaea, but flanked with Illumina overhang sequences. PCRs were performed in 25 μl reactions consisting of 12.5 μl of RedTaq ReadyMix (Sigma Aldrich) 200 nM of forward and reverse primers and 1 μl of template. DNA cycling conditions for all genes were 95 °C for 3 min followed by 28 or 31 cycles of 95 °C for 30 s; 57 °C for 30 s (except *amoA* AOA which was at 55 °C); 72 °C for 30 s and a final extension of 72 °C for 5 min). Amplicons were purified using Agencourt AMPure XP (Beckman Coulter) beads, before adding unique combinations of Nextera XT paired-end Indices via an 8-cycle PCR (reactions contained 25 μl of RedTaq ReadyMix, 5 μl each of unique Nextera XT Index, 5 μl of DNA (from cleaned PCR1). Amplicons were again purified using AMPure XP beads, quantified using a Quant-iT Picogreen dsDNA assay kit (Life Technologies) on a Nanodrop 3300 fluorospectrometer (Thermo Scientific) and pooled in equimolar concentrations. The quality of amplicon libraries were verified using a DNA 1000 kit on a 2100 Bioanalyzer (Agilent) before final pooled libraries were sequenced on the Illumina Miseq platform using a MiSeq reagent kit V3 (2 × 300 bp) at The Earlham Institute (formally The Genome Analysis Centre, Norwich, UK).

Sequence reads were de-multiplexed on the MiSeq platform and analysis was performed on the forward reads, following protocols described by (Dumbrell et al., 2016). The sequences were quality trimmed using Sickle (Joshi and Fass, 2011), with a min quality threshold of q20. Sequences were then error corrected with SPAdes (Nurk et al., 2013) using the BayesHammer algorithm (Nikolenko et al., 2013). The sequences were then de-replicated, sorted by abundance, and Operational Taxonomic Units (OTU) centroids were picked using VSEARCH (Rognes et al., 2016) at 97% similarity. Singleton OTUs were removed from the dataset, along with any chimeric sequences identified by both *de novo* and reference based chimera checking with UCHIME (Edgar et al., 2011). Taxonomy assignment for 16S rRNA sequences was

performed with the RDP Classifier (Wang et al., 2007). Non locus-specific OTUs were removed prior to statistical analyses.

Phylogenetic trees of translated amino acid sequences were constructed as previously described (Lansdown et al., 2016). Centroid sequences of the most abundant OTUs (comprising >99% of the sequencing reads for each gene) were aligned by codons using MUSCLE, in the MEGA6 program (Edgar, 2004; Tamura et al., 2013). Non-specific sequences detected during the alignment were discarded from all downstream analyses, and removed from OTU tables. Sequences were aligned with other known ammonia-oxidiser *amoA* sequences from the Fungene database (Fish et al., 2013) and from BLAST analyses (Altschul et al., 1990). Maximum likelihood trees were constructed on amino acid sequences, using the Le and Gascuel (2008) substitution model. Evolutionary rate differences between sites were modelled with a discrete Gamma distribution. The phylogeny was tested using 1000 bootstrap permutations.

2.5. Statistical analyses

OTU tables were imported into R (version 3.4.3) for statistical analyses (R Core Team, 2016). After discarding samples that had excessively small library sizes (i.e. per sample: <675 sequences for archaeal 16S rRNA gene, 11,773 sequences for bacterial 16S rRNA gene, 2928 sequences for AOA *amoA* gene, and 1190 sequences for AOB *amoA* gene), OTU tables were rarefied to an even depth with the "*vegan*" package (Oksanen et al., 2017). Compositional differences in the soil microbial communities were quantified using the Sørensen index (Baselga et al., 2017) and visualised using non-metric multidimensional scaling analyses (NMDS). PERMANOVA analyses were performed to test for compositional differences between different geologies, using 10,000 permutations. Differences in OTU richness of AOA and AOB in relation to geology were tested with negative binomial GLMs. Finally, putatively different functional groups of AOA and AOB were identified by grouping OTUs with identical *amoA* amino acid sequences (as identified through the phylogenetic analyses described previously). Differences in the abundances of these putative functional groups across geologies were then tested with multivariate negative binomial GLMs (Wang et al., 2007). Raw sequence data were submitted to the European Nucleotide Archive under accession number PRJEB28502.

3. Results

3.1. Soil physicochemical characteristics

There was no significant temporal variation ($P > 0.05$) of either SOC or SON across soils. However, clay soil had significantly lower concentrations of SOC and SON than Greensand and Chalk across the year ($P < 0.05$ in both cases), and were significantly more acidic than the near neutral pH Chalk (coef = 2.73, $t_{2,\ 6} = 17.75$, $P < 0.001$) and Greensand (coef = 2.22, $t_{2,\ 6} = 14.43$, $P < 0.001$) soils (Table 1). Turnover times of the SON pools were ca. three years for all geologies (Table 1). The C:N (g:g) ratios also differed across sites with near 10 in both clay and Greensand ($P > 0.05$), but higher (mean 12.6, $P < 0.001$) in Chalk soils (Table 1).

3.2. Mineralization and nitrification rates

Mean mineralization rates differed across geologies with rates of 5.9 and 8.3 μg N g^{-1} soil day^{-1} for Greensand and Chalk respectively, compared to 4.6 μg N g^{-1} soil day^{-1} in the more acidic clay soils throughout the year (Table 1, Fig. 2). Mineralization rates were significantly slower in the autumn compared to summer (coef = 1.61, $t = 3.35$, $P < 0.01$) and Spring (coef = 1.78, $t = 3.70$, $P < 0.001$), (although these differences were only statistically significant after accounting for geological variation). Ammonium pool turnover times in all soils across the year were <1 day (Fig. 2), indicating that the soil ammonium pools were highly dynamic throughout the year. In the clay soils in the summer, when soil ammonium concentrations were the lowest, ammonium turnover times were the slowest. In contrast, for the rest of the year in clay soils, when ammonium concentrations were higher, ammonium turnover times were faster. In the Chalk soils (in autumn), when ammonium concentrations were low, ammonium turnover times were slower, but increased in the winter when ammonium concentrations were higher. With Greensand soils, ammonium turnover times remained relatively slow throughout the year, and ammonium concentrations ranged between 1.15 and 2.23 μg N g^{-1} soil day^{-1}.

The soil nitrate pools were larger (8.1–29.4 μg N g^{-1} dry soil) than ammonium pools (0.4–4.3 μg N g^{-1} dry soil) (Table 2); and turnover times of nitrate pools were considerably longer (several days) than ammonium (fractions of a day) (Fig. 2). Turnover times of the soils' nitrate pools also showed temporal changes, with the longest turnover times in the autumn (Fig. 2). Across geologies, ammonium concentrations were greatest in the Chalk soils, where SON and SOC were also greatest (Table 1, Table 2). Temporally, ammonium concentrations were lowest in the autumn, (when nitrate was highest), yet differences in ammonium concentrations across months and geologies were not statistically significant ($P > 0.05$ in all cases) (Table 1, Table 2).

The ^{15}N reassimilation of ammonium into microbial cells (immobilisation) after its mineralization to ammonium gave only extremely low, often negative, and very variable rates between replicates, and were usually not significantly different ($P > 0.05$) from zero. Assimilation is generally only a small percentage (<10%) of nitrogen flow during microbial growth, compared to dissimilative metabolism (e.g. Pirt, 1975). Measurable remineralization of reassimilated ammonium by the soil microbiome after only a week-long incubation has been reported (e.g. Bjarnason, 1988), but our measurements after only one day incubation were highly unlikely to detect reassimilation as was also reported in grassland soils elsewhere within 48h (Braun et al., 2008). However, while more prolonged incubation is likely to detect reassimilation, it is also likely to enhance any enclosure effect rather than reflect *in situ* rates. Therefore, we do not consider immobilisation (reassimilation) data further in the current study, apart from noting that it is a very low and variable proportion of N flow in these soils compared to other processes.

Nitrification rates showed temporal variation (Fig. 2), with significantly slower rates in the autumn compared to summer (coef = 5.16, $t_{3,\ 35} = 2.32$, $P < 0.05$) and winter (coef = 5.04, $t_{3,\ 35} = 2.27$, $P < 0.05$) apart from in Greensand soils ($P = 0.50$), and correlated with lower soil ammonium concentrations for Greensand and Chalk soils, but not in the clay soils which had higher soil ammonium levels in the autumn than the summer. Across sites, the proportion of SON mineralized that was subsequently nitrified was (with the exception of Greensand) smallest in

Table 1
Soil organic carbon (SOC) and soil organic nitrogen (SON) concentrations, SON mineralization rate, SON pool turnover times, at 0–10 cm soil depth across geologies and sampling period (overall mean ± SE).

Site	pH	SOC (mg C g^{-1}soil)	SON (mg N g^{-1}soil)	C:N g/g ratio	Mean SON mineralization rate (μg N g^{-1} soil day^{-1})	SON pool turnover time (years)
Clay	4.9 ± 0.2	52.4 ± 6.62	5.38 ± 1.56	9.74 ± 0.3	4.6	3.2
Greensand	7.1 ± 0.3	74.64 ± 16.69	7.62 ± 1.56	9.8 ± 0.4	5.9	3.5
Chalk	7.6 ± 0.2	127.50 ± 1.41	10.11 ± 3.75	12.6 ± 4.5	8.3	3.3

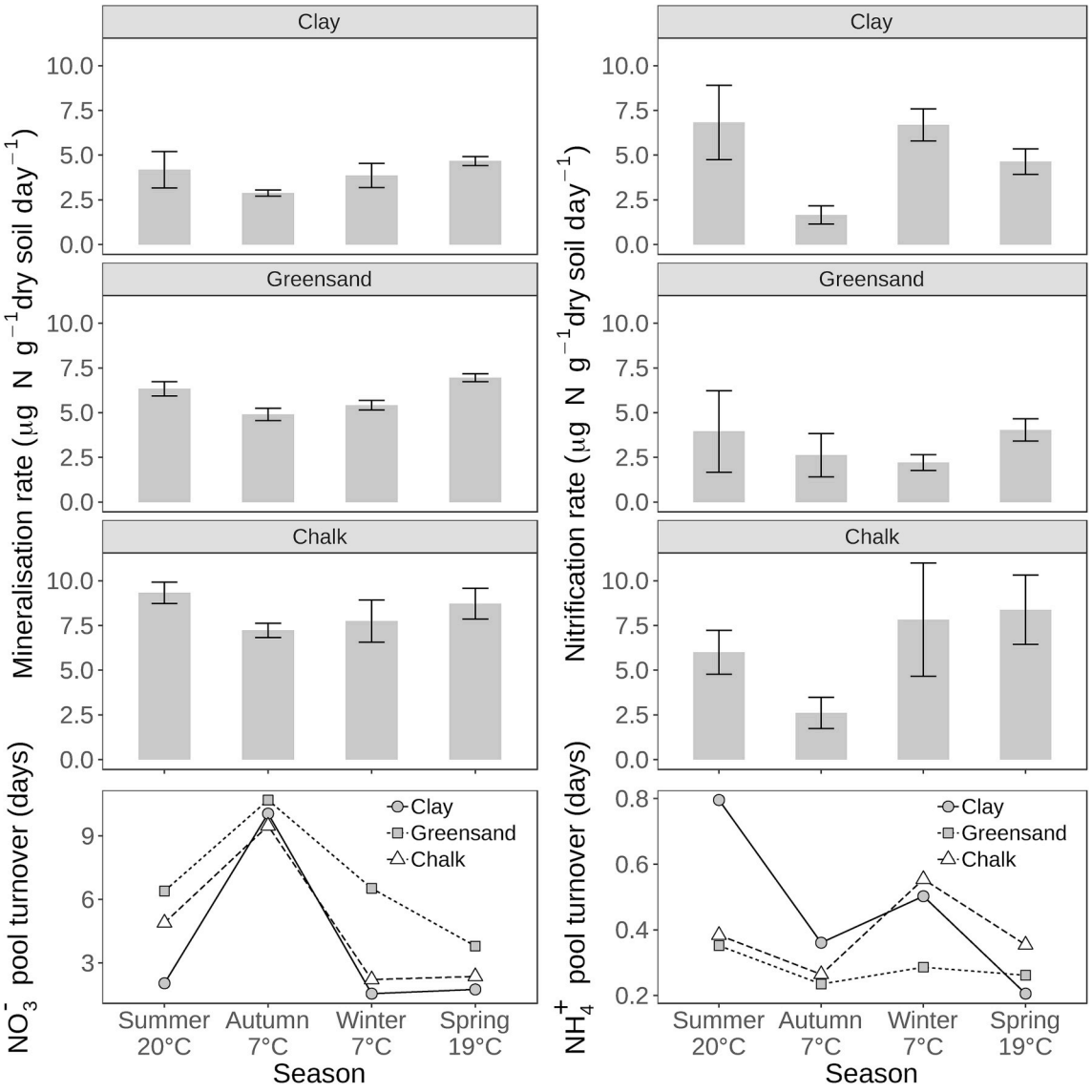

Fig. 2. The nitrification and mineralization rates, and nitrate and ammonium pool turnover times for soils on different underlying geologies.

Table 2
Soil ammonium, nitrate and phosphate concentrations, mineralization and nitrification rates across geologies and sampling period.

Site/Season	Substrate/Process (±SE)	Summer	Autumn	Winter	Spring	Mean
Clay	Nitrate (μg N g^{-1} dry soil)	13.90 ± 2.16	16.63 ± 0.98	10.36 ± 0.44	8.10 ± 1.17	12.25 ± 1.12
	Phosphate (μmol g^{-1} dry soil)	0.07 ± 0.01	0 ± 0.0	0.03 ± 0.02	0.86 ± 0.01	0.24 ± 0.01
	Ammonium (μg N g^{-1} dry soil)	0.36 ± 0.33	1.04 ± 0.05	1.94 ± 0.41	1.52 ± 0.06	1.22 ± 0.21
	Mineralization (μg N g^{-1} dry soil day^{-1})	4.19 ± 1.02	2.88 ± 0.17	3.86 ± 0.68	4.67 ± 0.25	3.9 ± 0.53
	Nitrification (μg N g^{-1} dry soil day^{-1})	6.82 ± 2.08	1.65 ± 0.51	6.69 ± 0.89	4.64 ± 0.71	4.95 ± 1.05
	Proportion of N mineralized then nitrified (%)	162	57	173	100	123
Greensand	Nitrate (μg N g^{-1} dry soil)	25.25 ± 1.77	28.02 ± 6.27	14.39 ± 2.03	15.30 ± 3.63	20.74 ± 3.43
	Phosphate (μmol g^{-1} dry soil)	0.06 ± 0.01	0 ± 0.0	0.6 ± 0.02	0.71 ± 0.09	0.34 ± 0.12
	Ammonium (μg N g^{-1} dry soil)	2.23 ± 2.18	1.15 ± 0.13	1.55 ± 0.34	1.82 ± 0.27	1.69 ± 0.73
	Mineralization (μg N g^{-1} dry soil day^{-1})	6.33 ± 0.40	4.90 ± 0.34	5.42 ± 0.27	6.96 ± 0.22	5.90 ± 1.23
	Nitrification (μg N g^{-1} dry soil day^{-1})	3.95 ± 2.28	2.62 ± 1.22	2.21 ± 0.44	4.03 ± 0.62	12.81 ± 1.14
	Proportion of N mineralized then nitrified (%)	62	53	40	58	53
Chalk	Nitrate (μg N g^{-1} dry soil)	29.37 ± 13.29	24.72 ± 0.80	17.31 ± 1.32	19.77 ± 4.96	22.79 ± 5.09
	Phosphate (μmol g^{-1} dry soil)	0.09 ± 0.01	0 ± 0.0	0.77 ± 0.05	3.88 ± 0.09	1.19 ± 0.04
	Ammonium (μg N g^{-1} dry soil)	3.58 ± 3.40	1.91 ± 0.19	4.29 ± 1.26	3.09 ± 0.21	3.22 ± 1.27
	Mineralization (μg N g^{-1} dry soil day^{-1})	9.33 ± 0.59	7.23 ± 0.40	7.74 ± 1.18	8.72 ± 0.86	8.23 ± 0.76
	Nitrification (μg N g^{-1} dry soil day^{-1})	6.00 ± 1.23	2.61 ± 0.75	7.82 ± 3.17	8.37 ± 1.93	24.80 ± 1.77
	Proportion of N mineralized then nitrified (%)	64	36	101	96	74

the autumn when both ammonium concentrations and nitrification rates were lowest, but not in the clay soils which had higher soil ammonium levels in the autumn than the summer (Table 2). On an annual basis, the average proportion of ammonia derived from N mineralization that was subsequently nitrified varied across sites from 123% in clay, but only 53% in Greensand and 74% in Chalk soils (Table 2).

3.3. 16S rRNA bacterial, archaeal, AOA and AOB communities

Distinct bacterial, archaeal, AOA and AOB communities were found in the 16S rRNA gene libraries in the less permeable clay soils, compared to the more permeable Chalk and Greensand soils, which were indistinguishable from each other (Fig. S1). PERMANOVA analyses revealed that communities were more similar within a geology than between geologies (AOA; pseudo-F = 26.98, $R^2 = 0.56$, $P < 0.001$; AOB; pseudo-F = 11.15, $R^2 = 0.35$, $P < 0.001$). Clay soils were also distinct in terms of ammonia oxidiser richness, being significantly lower in OTU richness than Greensand and Chalk soils for both AOA, (clay-Greensand; coef = 0.99, $z = 3.62$, $P < 0.001$, clay-Chalk; coef = 0.21, $z = 2.66$, $P < 0.01$), and AOB, (clay-Greensand; coef = 0.28, $z = 5.64$, $P < 0.001$, clay-Chalk; coef = 0.62, $z = 3.47$, $P < 0.001$) (Fig. S1E –F). However, OTU richness of AOA and AOB, did not differ significantly across sampling months ($P > 0.05$ in all cases).

Within archaeal 16S rRNA libraries, AOA were the most abundant with *Nitrososphaera* accounting for 37.4% of total observed sequences, and the vast majority found in Chalk (56.7%) and Greensand (63.7%) compared to clay (1.2%) soils (Fig. S2). In addition to AOA, a further seven genera, which although generally more abundant in clays, comprised <0.5% of the Archaeal library (Fig. S2), leaving >62% of OTUs that could not be confidently assigned to a particular archaeal genus. AOA 16S rRNA gene abundance did not differ significantly throughout the year ($P > 0.05$ in all cases) (Fig. S2). Within the *Nitrososphaera,* 12 OTUs were identified (Fig. S3). The two most abundant OTUs (OTU9, OTU6) represented 19.4% and 8.8% of observed archaeal 16S rRNA sequences, respectively), and were 94–95% similar to *Nitrososphaera viennensis*.

Within the bacterial 16S rRNA libraries, the most abundant bacterial taxa were Acidobacteria (7.1%) followed by Actinobacteria (5.8%) and Betaproteobacteria (5.0%), with 11.1% of sequences corresponding to unclassified bacteria. Eight genera (representing <1.4% of observed bacterial sequences) showed temporal abundance shifts (Fig. S4). A total of 67 genera changed across geology, (collectively representing 31.8% of the bacterial 16S rRNA library) (Fig. S5). AOB represented only <0.06% of the total observed bacterial 16S rRNA gene sequences, and all were *Nitrosospira* spp. Moreover, only two AOB OTUs were identified within the bacterial 16S rRNA library and had 99% identity to *Nitrosospira multiformis* (OTU1) and *Nitrosospira lacus* (OTU2) (Fig. S6).

3.4. AOA and AOB amoA gene analysis

Generally, AOA *amoA* gene abundances were an order of magnitude greater than AOB across geology and sampling period (Fig. 3). AOA *amoA* gene abundances ranged from 1.7×10^6 to 1.8×10^8 copies g^{-1} dry soil compared to AOB 6.1×10^5 to 1.8×10^7 copies g^{-1} dry soil. AOA and AOB *amoA* gene abundance differed significantly between geologies: clay contained significantly fewer *amoA* genes on average than Greensand ($P < 0.01$ for both AOA and AOB). However, only for the AOA *amoA* abundance did all three sites differ significantly from each other (Tukey HSD test; $P < 0.05$ for all comparisons). Clay harboured the lowest AOA abundances (1.7×10^6 to 1.5×10^7 *amoA* genes g^{-1} dry

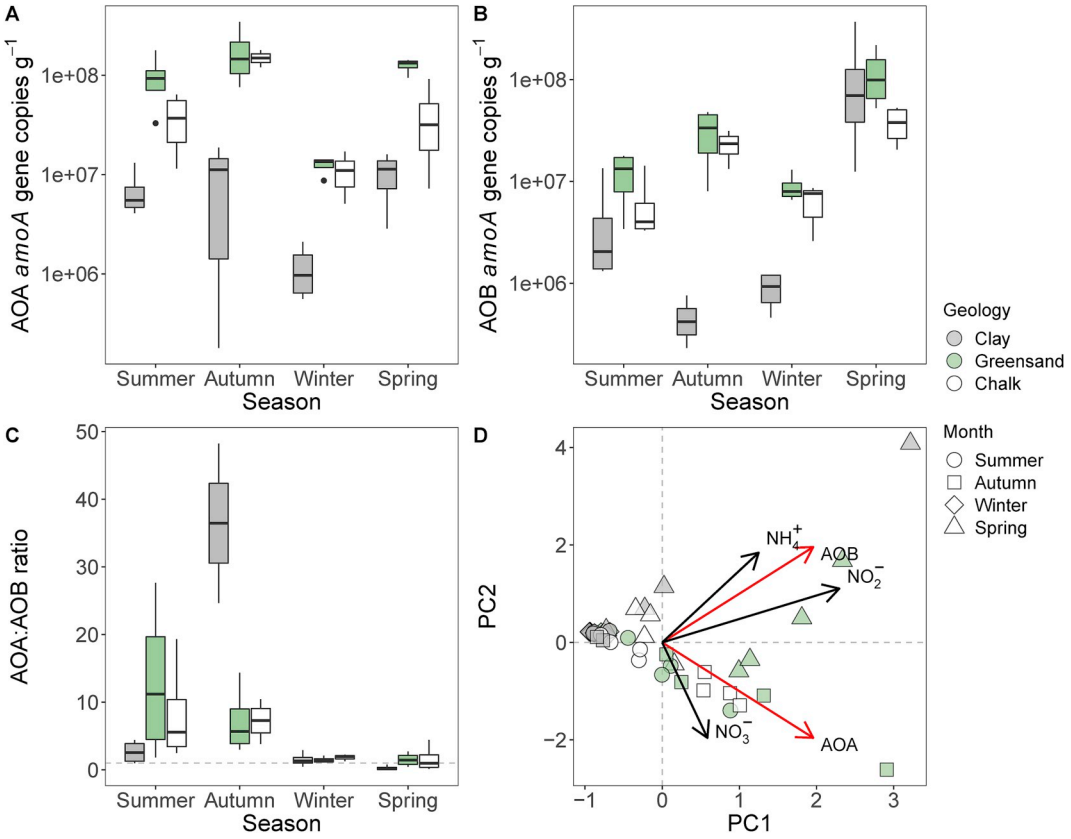

Fig. 3. The abundance of ammonia oxidiser *amoA* genes. Panels A and B show the abundance of ammonia oxidising Archaea and Bacteria respectively, across sampling months and underlying geologies; panel C shows the ratio of ammonia oxidising Archaea to Bacteria; panel D shows PCA correlation analysis of AOB and AOA abundance (as measured by qPCR), with specific environmental factors (geology, season) and vectors (soil ammonium, nitrite and nitrate concentrations) overlaid.

soil), compared to 1.8×10^7–1.8×10^8 *amoA* genes g^{-1} dry soil for Greensand (~15 fold higher than clay), and 1.6×10^7 to 1.2×10^8 *amoA* genes g^{-1} dry soil) for Chalk soils (Fig. 3A). AOB followed a similar pattern to AOA, with clay having the lowest AOB abundances, followed by Chalk, and Greensand soils having the highest abundances (Fig. 3B). However, only clay and Greensand soils had significantly different AOB abundances over the course of a year (relative to clay; coef = 1.04, z = 3.29, $P < 0.01$). AOA *amoA* abundances were significantly lower ($P < 0.001$, for all comparisons) in winter (Fig. 3A), than at any other time, and were significantly higher in the autumn than summer and winter ($P < 0.05$ for both comparisons), but not spring. AOB showed markedly different temporal dynamics, with their abundance peaking in spring at all sites, (relative to Spring; coef < −1.87, $z < -5.06$, $P < 0.001$, for all comparisons) (Fig. 3B, Table S1).

Across geologies and sampling period, the ratio of AOA to AOB *amoA* usually favoured AOA (1st quartile = 1.12, median = 5.71, 3rd quartile = 5.37; Fig. 3C), but ranged from 0.03 (favouring AOB) to 48.2 (favouring AOA). In all sites, the ratio remained close to 1 during winter and spring, indicating approximately equal proportions of AOA and AOB. However, in summer and autumn, the ratio increased above 1 in all geologies, particularly so in clays during autumn (between 24.7 and 48.2, median = 36.5), due to there being an order of magnitude fewer AOB than in the same soils during the rest of the year. Conversely, in Chalk and Greensand soils this increase was due to an increase in AOA populations.

Changes in AOA abundance were found to positively correlate with changes in nitrate concentration (coef = 1.70, z = 2.22, $P < 0.05$), whereas AOB abundance correlated with ammonium coef = 2.00, z = 4.62, $P < 0.001$) and nitrite (coef = 63.34, z = 4.89, $P < 0.001$) concentrations (Fig. 3D). Statistical differences in AOA and AOB abundance across soils throughout the year (based on *amoA* qPCR data), and their correlation with overall archaeal and bacterial community size (as determined by 16S rRNA qPCR), were tested for using negative binomial generalised linear models (GLMs) in relation to ammonium and nitrate concentrations. We found that when ammonium concentrations were low, AOA were more abundant than AOB across soils. However, when nitrate concentrations were low, AOB were more abundant than AOA (Fig. S7).

Phylogenetic analysis of AOA *amoA* genes showed that all the AOA OTUs clustered within the family Nitrososphaeraceae (Fig. 4), specifically most closely to *Nitrososphaera gargensis* and *Nitrosocosmicus franklandus*. When OTUs were translated to their amino acid structure, the AOA formed three OTU groups with distinct amino acid sequences (Fig. 4, Table S2). OTU Group 1 was significantly more abundant in both Greensand (coef = 4.60, $z = 12.17$, $P < 0.001$) and Chalk (coef = 4.47, z = 11.83, $P < 0.001$), irrespective of month, compared to clay soils, whereas Group 3 showed the opposite pattern, being more abundant throughout the year in clay compared to Chalk (coef = −7.90, z = −12.03, $P < 0.001$) or Greensand (coef = −7.50, $z = -12.71$, $P < 0.001$) (Fig. S8A). Group 2 was more abundant on average in clay soils throughout the year compared with Greensand (coef = −3.93, z = −3.81, $P < 0.001$), or Chalk soils (coef = −1.99, $z = -2.16$, $P < 0.05$), except in autumn where it was more abundant in Chalk soils (coef = 3.43, $z = 2.66$, $P < 0.01$).

Phylogenetic analysis of AOB *amoA* genes showed that all AOB OTUs clustered within the genus *Nitrosospira* (Fig. 5). AOB OTUs translated into 6 unique amino acid variants (Fig. 5, Table S3). Some of these groups displayed similar patterns as AOA, being differentially abundant in clay versus Chalk and Greensand soils. Groups 2 and 5 were both more abundant in the Greensand (Group 2; coef = 5.84, $z = 7.30$, $P < 0.001$,

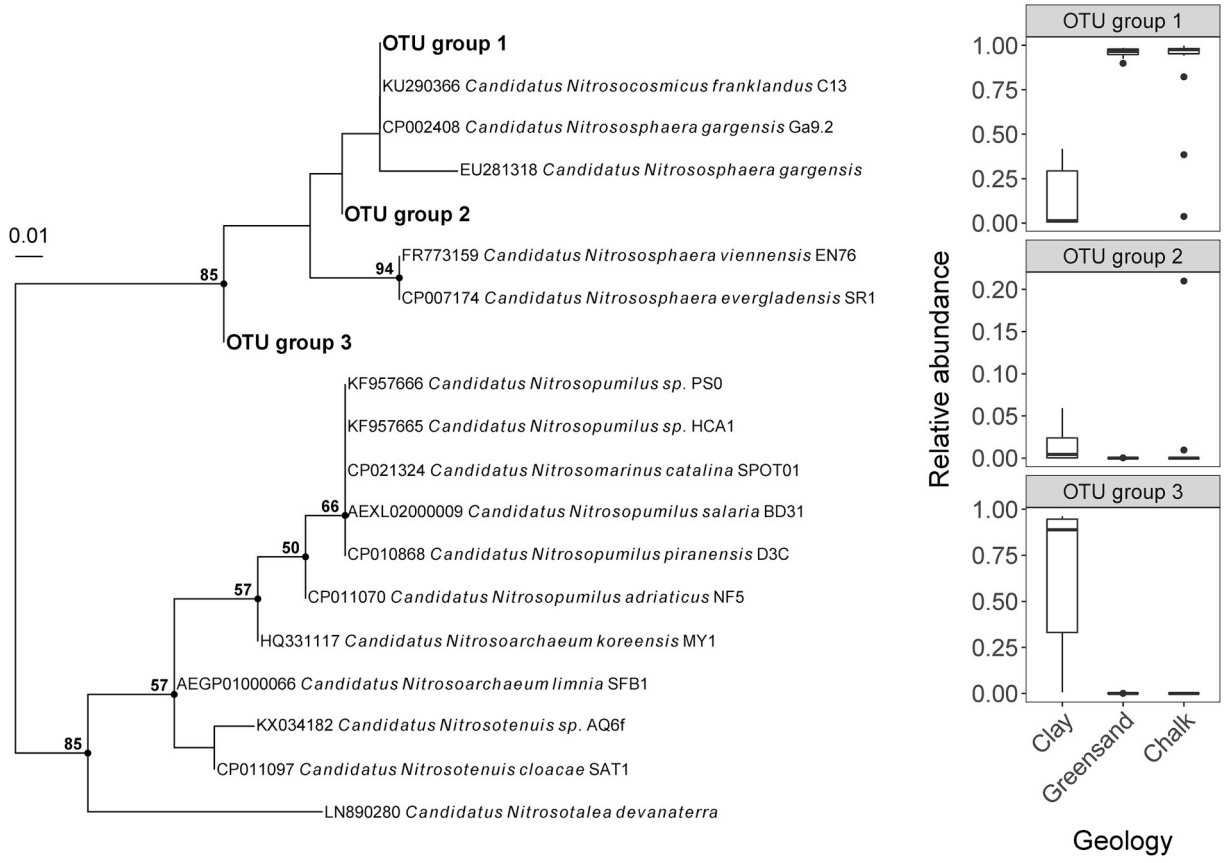

Fig. 4. A phylogenetic tree based on ammonia oxidising archaeal AmoA amino acid sequences. OTU groups consist of multiple OTUs with identical amino acid sequences, and additional AmoA sequences were obtained from BLAST and Fungene searches. The right-hand panels show the relative abundances of OTUs in OTU groups 1, group 2, and group 3 across the three geologies.

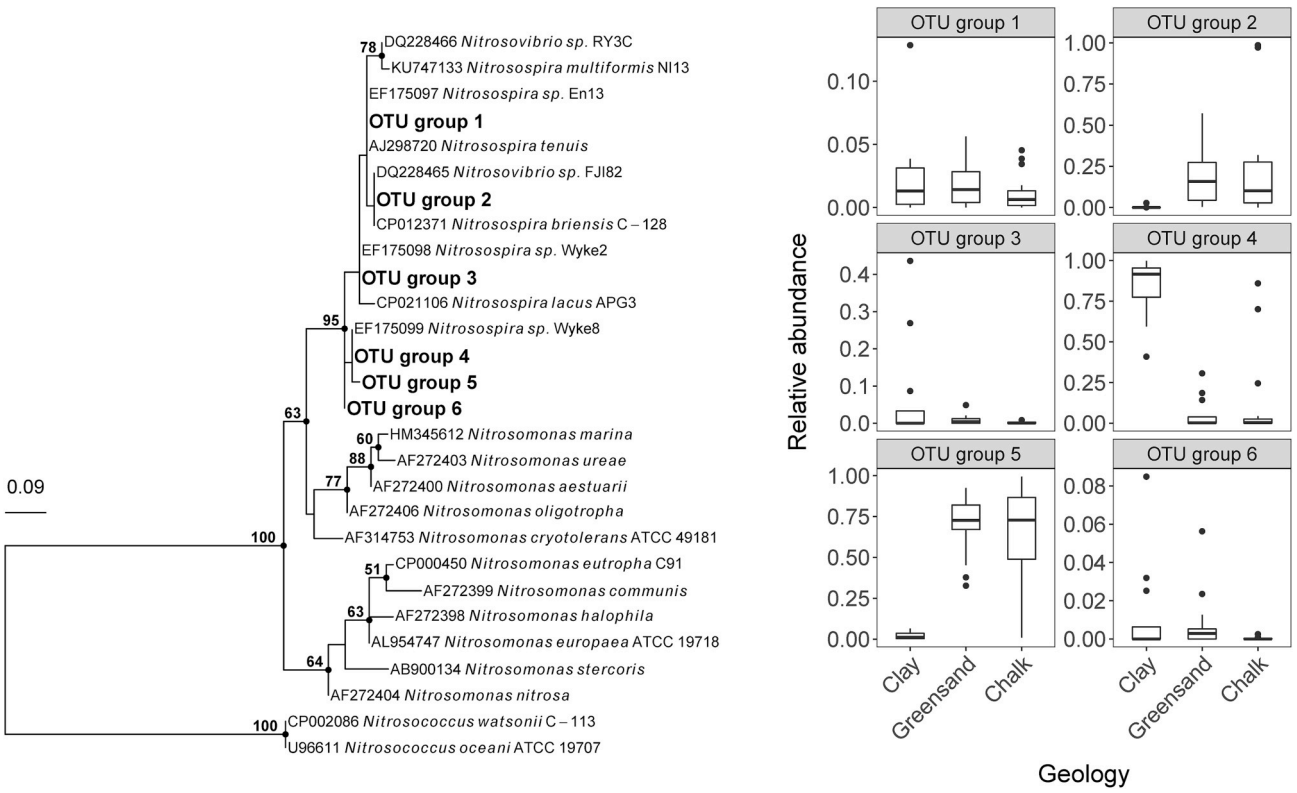

Fig. 5. A phylogenetic tree based on ammonia oxidising bacterial AmoA amino acid sequences. OTU groups consist of multiple OTUs with identical amino acid sequences, and additional AmoA sequences were obtained from BLAST and Fungene searches. The relative abundances of OTUs in each of the OTU groups are shown in the right-hand panels.

Group 5; coef = 4.05, $z = 6.91$, $P < 0.001$) and Chalk soils (Group 2; coef = 4.31, $z = 5.37$, $P < 0.001$, Group 5; coef = 4.13, $z = 7.04$, $P < 0.001$) than clay soils, though only Group 2 showed temporal changes, being more abundant on average in spring (coef = 1.98, $z = 2.41$, $P < 0.05$). Groups 3 and 4 displayed the reverse pattern as they were more abundant in clay than Greensand (Group 3; coef = −2.08, $z = -2.58$, $P < 0.05$, Group 4; coef = −6.66, $z = -8.01$, $P < 0.001$) or Chalk soils (Group 3; coef = −3.28, $z = -4.03$, $P < 0.001$, Group 4; coef = −1.62, $z = -2.24$, $P < 0.05$). Group 6 was more abundant in Greensand soils compared to clay or Chalk soils (coef = 1.95, $z = 2.41$, $P < 0.05$), except during the spring, when their abundance in clay soils was significantly higher than in Chalk (coef = −4.10, $z = -3.46$, $P < 0.001$) and Greensand soils (coef = −3.34, $z = -3.26$, $P < 0.01$).

4. Discussion

In grassland soils the rate of organic mineralization as a supply of ammonium in relation to AOA and AOB communities has not been fully investigated. In this study, N mineralization rates measured in grassland soils in the Hampshire Avon catchment (southern United Kingdom), were similar to those reported for other European grasslands (e.g. 5.3 ± 0.1 μg N g^{-1} day^{-1}) (Braun et al., 2008). Here, temporal differences in N mineralization were found, with lower rates in the autumn than at other times of the year that may be due to changes in the physiological response of the existing microbial community to some environmental or edaphic factor.

Previous work showed that increasing mean annual temperatures may increase net N mineralization in grassland soils (Smith et al., 2002; Hutchinson, 1995; Zhang et al., 2012). In tropical soils, temperature affects mineralization rates (Myers, 1975). In our study, temperatures ranged between ~20 °C (spring/summer) to ~7 °C (autumn/winter). It is therefore possible that the lower autumn/winter temperatures affected ammonifier physiology and a period of adaption to these colder temperatures was required. King and Nedwell (1984) demonstrated a two month lag adaption period to temperature occurred with sediment nitrate reducers. Alternatively, other soil factors (e.g. pH) may have affected ammonifier physiology. Indeed mean N mineralization rates differed across geologies with between 1.5 and 2-fold faster rates in the near neutral Greensand and Chalk soils (pH 7.2 and 7.6 respectively) compared to the more acidic clay (pH 5.5) soils throughout the year, despite climatic conditions for the region being typical during the sampling period. Other studies however, have shown that soil pH does not affect mineralization rates (Dancer et al., 1972).

Changes in the size or composition of the heterotrophic or mixotrophic microbial communities may also explain the differences in mineralization rates. However, since the 16S rRNA archaeal community structure was found to be temporally stable, and only <1.4% of bacterial abundances changed throughout the year this is unlikely. Heterotrophic and mixotrophic AOA have also been reported and may have contributed to the observed N mineralization rates (Hallam et al., 2006; Walker et al., 2010; Prosser and Nicol, 2012; Zhalnina et al., 2012). However, when mineralization rates were slowest in the autumn, AOA *amoA* gene abundances were highest (in Greensand and Chalk) than the rest of the year. Other studies have shown contrasting findings whereby the abundance of AOA *amoA* genes correlate both positively and negatively with total soil C and N, suggesting the response of AOA to C and N is very complex (Zhalnina et al., 2012; Xie et al., 2014; Liu et al., 2018). Here, AOA did not correlate with total soil C.

Recently, it was shown that N mineralization rates increased as organic N uptake exceeded microbial growth N demand (Zhang et al., 2019). Here, we determined what proportion of the SON mineralized to ammonium was subsequently nitrified. In general, across geology this proportion was smallest in the autumn (for clay and Chalk soils) when both ammonium concentrations and nitrification rates were lowest. On an annual basis the average proportion of N mineralized that was subsequently nitrified although varied, was highest in the clay soils. This

might suggest in clay an additional, yet unknown input of ammonium into nitrification, over and above that derived from SON breakdown, whereas in Greensand and Chalk only part of the mineralized nitrogen was nitrified.

One possible explanation for this additional N input to soil is atmospheric deposition of N. However, atmospheric inputs of total nitrogen in the area of the Hampshire Avon catchment are about 12 kg ha^{-1} yr^{-1} (based on CEH CBED model data http://www.pollutant deposition.ceh.ac.uk/data), compared to 2–3 tonnes N ha^{-1} yr^{-1} by SON mineralization, so atmospheric deposition would seem unlikely to account for the difference, and moreover would not explain the differences observed between geologies. In the Greensand and Chalk soils, the proportion of N mineralized but not nitrified may have also been lost by export of N by leaching or as ammonia emissions across the catchment but again would not explain the differences observed between geologies.

Within the Hampshire Avon sub-catchments, all the grassland sites were grazed (albeit on a grazing rotation), however, the distribution of arable and livestock farming varied across sites. Within the clay grasslands, dairy farming is supported whilst arable agriculture represents a larger proportion of land use in the Greensand and Chalk sites; with up to 55% of Chalk sites being arable and sheep grazing and pig production as minority uses (Heppell et al., 2017). Thus, grazing by dairy cattle are likely to have higher urine/faecal inputs in the clay soils compared to the sheep grazed Greensand and Chalk soils, which may offer one possible explanation for the imbalance between mineralization and nitrification. However, N input to soils via urine/faecal addition was not measured in our study.

Nitrification rates were similar to those reported elsewhere (e.g. 6–170 μg N g^{-1} dry soil day^{-1}) (Mørkved et al., 2007). In other studies on grassland soils, nitrification rates showed similar trends as N mineralization rates (Zhang et al., 2012), but in our study, although nitrification rates varied spatially and temporally, nitrification and mineralization rates showed similar general trends. Changes in temperature may also affect nitrification rates (Myers, 1975) and the proportion of nitrification by AOA (Ouyang et al., 2017). In our study, slower nitrification rates in the autumn (for clay and Chalk) generally corresponded to higher AOA *amoA* abundances. At this time, ammonium concentrations were also lower and ammonium pool turnover times were generally fastest (with the exception of clay soils in the spring and Greensand between autumn to spring), and thus competition for ammonium would be particularly intense. In general, ammonium pool turnover times in all soils across the sampling period were <1 day whilst turnover times of nitrate pools were considerably longer (several days) indicating that the soil ammonium pools were highly dynamic throughout the year compared to soil nitrate.

We observed that AOA were generally more abundant than AOB with the exception of clay soils where both AOA and AOB were low in abundance. Our findings suggest that AOA were potential drivers of ammonia oxidation (particularly in the Greensand and Chalk soils). Jia and Conrad (2009) showed that potential nitrification correlated with AOA abundance despite high ammonium concentrations favouring AOB. However, this does not explain the lower AOA (and AOB) *amoA* gene abundances found in the clay soils when ammonium concentrations increased in the winter and the significantly lower in OTU richness with the clays than the Greensand and Chalk soils throughout the year.

Although AOA and AOB co-exist in the environment, they respond differently to environmental factors and there is evidence of niche differentiation among ammonia oxidisers in grasslands (Erguder et al., 2009; Prosser and Nicol, 2012; Li et al., 2015; Ouyang et al., 2016; Hink et al., 2018). Soil pH is an important factor in shaping ammonia oxidiser communities (Gubry-Rangin et al., 2011; Lehtovirta-Morley et al., 2011, 2014; Hu et al., 2013, 2015, 2014). Other studies showed that AOB (not AOA) positively correlate with soil pH (Liu et al., 2018). However, the underlying mechanisms of soil pH in shaping the ammonia oxidiser community are complex, with direct and indirect pH-associated influencing factors (Hu et al., 2015). In the more acidic clay soils, the soil water equilibrium will favour NH_4^+ ($pK_a = 9.24$), whereas in Greensand and Chalk soils the equilibrium will move towards ammonia gas (NH_3), albeit such differences are very small. In this context, Greensand and Chalk soils would likely be more susceptible to ammonia loss, favouring AOA over AOB. Interactions with other soil parameters may also be occurring. For example in clay soils, ammonium may adsorb to clay minerals (Hink et al., 2018), reducing substrate availability. We found that ammonium turnover time across soils was highly dynamic throughout the year. Phosphate limitation may also have been an important factor. In our study, phosphate concentrations were between 1.4 and 5 fold lower in the clay soils than in the other soils (Table 2). However, Hertfort et al. (2007) demonstrated a positive correlation between crenarchaeotal 16S rRNA gene copies and phosphate concentration.

Whilst we found that AOA were more abundant than AOB, spatially and temporally, we cannot attribute the nitrification rates measured here to either AOA or AOB. Furthermore, *Nitrospira* performing comammox (complete ammonia-to-nitrate oxidation) and their co-occurrence with AOB in the environment (Daims et al., 2015; Hu and He, 2017; Palomo et al., 2018) may also be contributing to the observed nitrification rates. AOA communities were entirely Nitrososphaeraceae (specifically *Nitrososphaera gargensis* and *Nitrosocosmicus franklandus*), and comprised ~37% of total observed archaeal 16S rRNA sequences. Phylogenetic analyses placed the OTUs observed entirely within the *Nitrososphaera* lineage as defined by Alves et al. (2018). In particular, *amoA* OTU group 2, which shared a common amino acid structure, fitted well within the α-subclade, whilst the exact positions of the other two OTU groups are less clear without further phylogenetic analyses. The dominance of *Nitrososphaera* in these grassland soils reflects similar findings in soils elsewhere (Leininger et al., 2006; He et al., 2007; Jiang et al., 2014; Liu et al., 2018).

AOA and AOB niches have also been defined by ammonium concentrations, with low ammonium environments selecting for AOA (Leininger et al., 2006; Martens-Habbena et al., 2009; Di et al., 2010). If AOA and AOB utilise ammonia with equal efficiency, AOA will only dominate activity if AOA:AOB is > 10 (Prosser and Nicol, 2012). Here, the ratio of AOA:AOB *amoA* genes tended to favour AOA, particularly in the summer and autumn, where the ratio rose to a maximum of 48.25. However, during winter and spring, the AOA:AOB remained close to 1, indicating approximately equal abundances of these two groups. Other studies on soils, found AOA:AOB *amoA* gene ratios ranged from 3.1 to 91.0 (Liu et al., 2018), 231 in unfertilised soils (Leininger et al., 2006), and 17 to >1600 in semiarid soils (Adair and Schwartz, 2008). In our study, the AOA:AOB negatively correlated with ammonium concentration and elsewhere it has been shown that AOA predominated in soil microcosms until high ammonium concentrations were added (Verhamme et al., 2011). However, other studies on grassland soils showed that addition of N does not necessarily affect AOA abundance (Chen et al., 2014).

Differences in substrate affinity for ammonium between AOA and AOB may explain these findings, as AOA have lower K_s and higher μmax values than AOB and will outcompete AOB for ammonia at all concentrations (Prosser and Nicol, 2012). However, contrasting substrate affinities does not explain that similar K_s values have been reported in some AOB (e.g. *Nitrosomonas europaea* and *Nitrosopumilus maritimus*) (Kits et al., 2017; Hink et al., 2018) and other AOA. Furthermore, differences in substrate affinities between AOA and AOB does not explain that AOA rather than AOB favour low ammonium environments such as unfertilised soils (Leininger et al., 2006; Di et al., 2010; Gubry-Rangin et al., 2010; Verhamme et al., 2011; Hink et al., 2017). In addition, whilst some AOA may be inhibited by high ammonium concentrations, several AOA isolates such as *Candidatus* Nitrosocosmicus species can grow in up to 100 mM ammonium (Jung et al., 2016; Lehtovirta-Morley et al., 2016; Sauder et al., 2017) and archaeal *amoA* genes have been detected in reasonably high ammonium concentrations (i.e. 10-18 mM, Treusch et al., 2005; Park et al., 2006). However, K_s values are poor

measures of affinity (Button, 1993; Nedwell, 1999), and a more robust measure of affinity is given by specific affinity a_A, which is equivalent to μ_{max}/K_s. The higher the value of a_A, the better is the affinity for the substrate. Using the values for μ_{max} and K_s for typical growth rates of AOA and AOB given by Prosser and Nicol (2012); the a_A for AOA is 5.28 l μmol^{-1} h^{-1} but only 0.002 l μmol^{-1} h^{-1} for AOB, showing that AOA have some 2600-fold higher affinity for ammonium than AOB. Indeed, the growth rate (μ) of AOA would exceed that of AOB at all soil ammonium concentrations measured. It can also be argued, that slow rates of ammonium supply result in low ammonium concentrations *in situ* and therefore substrate affinity is more likely to be controlling AOA and AOB competition. This suggests that in these grasslands, AOA are driving ammonia oxidation when ammonium supply rate is low.

5. Conclusions

In conclusion, the 16S rRNA bacterial and archaeal communities, and the AOA and AOB communities in clay were clearly distinct from those in the Chalk and Greensand soils. In general, AOA (*Nitrososphaera*) were more abundant than AOB across sites and sampling time (with the exception of clay soils in the spring, when AOB were dominant). Temporal differences in mineralization and nitrification rates across geology were also found, with lows during autumn which corresponded to higher AOA *amoA* gene copies (in Greensand and Chalk soils) supporting the idea that AOA are driving ammonia oxidation when ammonium supply rate is low. Here, changes in AOA abundance positively correlated with nitrate concentration, whereas AOB abundance correlated with ammonium and nitrite concentrations. These findings provide a better understanding of the drivers of soil N cycling for the 7.5 million ha of grassland in the UK where maintenance of soil fertility, carbon stocks and prevention of undesirable N loss are crucial to ecosystem function.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.107725.

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