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Oxygen preference of deeply-rooted mesophilic thaumarchaeota in forest soil

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ABSTRACT

Soil microorganisms require metabolic strategies to cope with the significant variations in oxygen availability that occur in soil over a range of time scales. Characterised ammonia-oxidisers within Thaumarchaeota Groups 1.1a and 1.1b are aerobic, but the oxygen preference and metabolic potential of deeply-rooted Thaumarchaeota remain unknown, with several studies providing evidence for both aerobic and anaerobic metabolisms. This study therefore aimed to determine the influence of oxygen on the mesophilic Group 1.1c and Group 1.3 thaumarchaeotal community in controlled microcosm experiments conducted using oxic and anoxic Scottish pine forest soils, incubated under oxic or anoxic conditions. While we expected more anaerobic growth metabolism in those peatland soils, we demonstrated growth of multiple aerobic clusters within Group 1.1c Thaumarchaeota and anaerobic growth within Group 1.3 Thaumarchaeota. These findings extend our understanding of the physiology of deeply-rooted mesophilic Thaumarchaeota and provide the first detailed qualitative and quantitative assessment of their growth in soil.

1. Introduction

Determining the potential of uncultivated soil microorganisms for aerobic and anaerobic metabolism is important for an understanding of ecosystem function in environments with variable oxygen availability (Laanbroek, 2010; Offre et al., 2013). Oxygen availability is influenced in terrestrial ecosystems by soil depth and water content and oxygen has been advanced as a critical environmental factor leading to niche specialisation and evolution of different soil microorganisms, including Haloarchaea (Nelson-Sathi et al., 2012) and Thaumarchaeota (Ren et al., 2019).

Microorganisms belonging to the Nitrososphaeria class within the Thaumarchaeota phylum perform the first step of nitrification, ammonia oxidation, which is of critical ecosystem importance. However, deeply rooted Thaumarchaeota are known to be non-ammonia oxidisers (Weber et al., 2015; Ren et al., 2019) and their metabolism remains underexplored, with availability of a few genomes and a single enrichment culture (Gubry-Rangin et al., 2018; Kato et al., 2019). A recent timeline of thaumarchaeotal diversification associated with environmental specialisation indicated that the first great oxygenation event (~2.3 My ago) coincided strongly with the appearance of the last ammonia oxidiser ancestor (Ren et al., 2019), suggesting that oxygen has been a key driver for thausmarchaeotal diversification. Other environmental characteristics, such as pH (Gubry-Rangin et al., 2015), influenced further niche specialisation and evolution of terrestrial ammonia-oxidising Thaumarchaeota.

Most thaumarchaeotal ecological analyses have focused on ammonia oxidising archaea (AOA) rather than on non-ammonia oxidisers (non-AOA) within this phylum, despite their widespread distribution in soils, sediments and hot springs (Rinke et al., 2013; Beam et al., 2014; Lin et al., 2015; Vico-Oton et al., 2016; Anantharaman et al., 2016; Hua et al., 2018). In particular, the presence of Group 1.1c Thaumarchaeota has been reported in many acidic soils (Vico Oton et al., 2016), including oxic top soils with relatively low matric potential that supports aerobic processes such as ammonia oxidation (Kemnitz et al., 2007; Lehtovirta et al., 2009; Auguet et al., 2010) and in association with ectomycorrhizas and plant roots (Sliwinski and Goodman, 2003; Bomberg et al., 2011; Mushinski et al., 2019) that may release oxygen (Hupfer and Dollan et al., 2003). There is also evidence that Group 1.1c Thaumarchaeota are present in anoxic environments, such as water-saturated

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bog soils (Juottonen et al., 2008; Stopnišek et al., 2012) and peat soils, where their abundance increases with depth (Bomberg et al., 2010; Basiliko et al., 2013; Hawkins et al., 2014; Lin et al., 2014). These environmental surveys suggest the existence of specialisation for oxygen tolerance and the potential for aerobic and anaerobic growth. A phylogenetic analysis of deeply-rooted Thaumarchaeota associated with ancestral reconstruction of several environmental preferences (Vico-Oton et al., 2016) indicated niche specialisation with respect to soil water content in the 12 defined non-AOA Thaumarchaeota phylogenetic clusters and, by inference, potential adaptation to distinct oxygen conditions. In particular, the four most abundant phylogenetic clusters (named clusters G3.1, GC1/GC2, GC3 and GC5) (see Fig. 1) are present in water-logged anoxic soils, suggesting anaerobic growth in these environments, while two of these clusters (GC1/GC2 and GC5) are also abundant in oxic environments, suggesting aerobic metabolism. All other less abundant non-AOA thaumarchaeotal clusters can be found in diverse environments, ranging from oxic to completely anoxic ones.

These metabolic predictions are reinforced by the existence of both potential aerobic and anaerobic metabolic pathways in non-AOA Thaumarchaeota metagenomes that have been recovered from anoxic peat soil (Lin et al., 2015), acidic geothermal springs (Beam et al., 2014) and hot spring sediment samples (Hua et al., 2018), affiliating respectively to Group 1.1c, Group 1.1d/1.1e and pSL12 lineages. This is consistent with evidence that most Aigarchaeota (sister group of Thaumarchaeota) are facultative anaerobes, with one recently diverged clade of closely related Aigarchaeota showing genomic evidence for a metabolic switch to a strict anaerobic lifestyle (Hua et al., 2018). The only deeply-rooted Thaumarchaeota isolate originates from a terrestrial acidic hot spring and has an anaerobic metabolism (Kato et al., 2019).

Previous studies therefore suggest the existence of both aerobic and anaerobic growth within non-AOA Thaumarchaeota and growth of some Group 1.1c Thaumarchaeota was previously demonstrated in oxic soils (Weber et al., 2015). However, the lack of cultivated representatives in most of these phylogenetic clusters severely limits current knowledge of their oxygen metabolism. Therefore, the aim of this study was to determine the oxygen preference of some of the most abundant soil non-AOA Thaumarchaeota in temperate soils and to test the potential for aerobic and anaerobic of these organisms. We hypothesised that growth of deeply rooted non-AOA Thaumarchaeota would vary with oxygen availability, with predicted growth of the two most abundant non-AOA Thaumarchaeota clusters (clusters GC1/GC2 and GC5) under both aerobic and anaerobic conditions, while the two next more abundant clusters (clusters G3.1 and GC3) would grow under anaerobic conditions (Vico Oton et al., 2016) (Fig. 1). This hypothesis was tested by investigating growth of non-AOA Thaumarchaeota from non-saturated shallow and deep wet regions of a pine forest soil, representing oxic and anoxic horizons, respectively, each incubated under oxic and anoxic conditions. Pine forest soil was selected on the basis of the previous study by Weber et al. (2015), which demonstrated the ability of Group 1.1c to grow in microcosms constructed with pine forest soil.

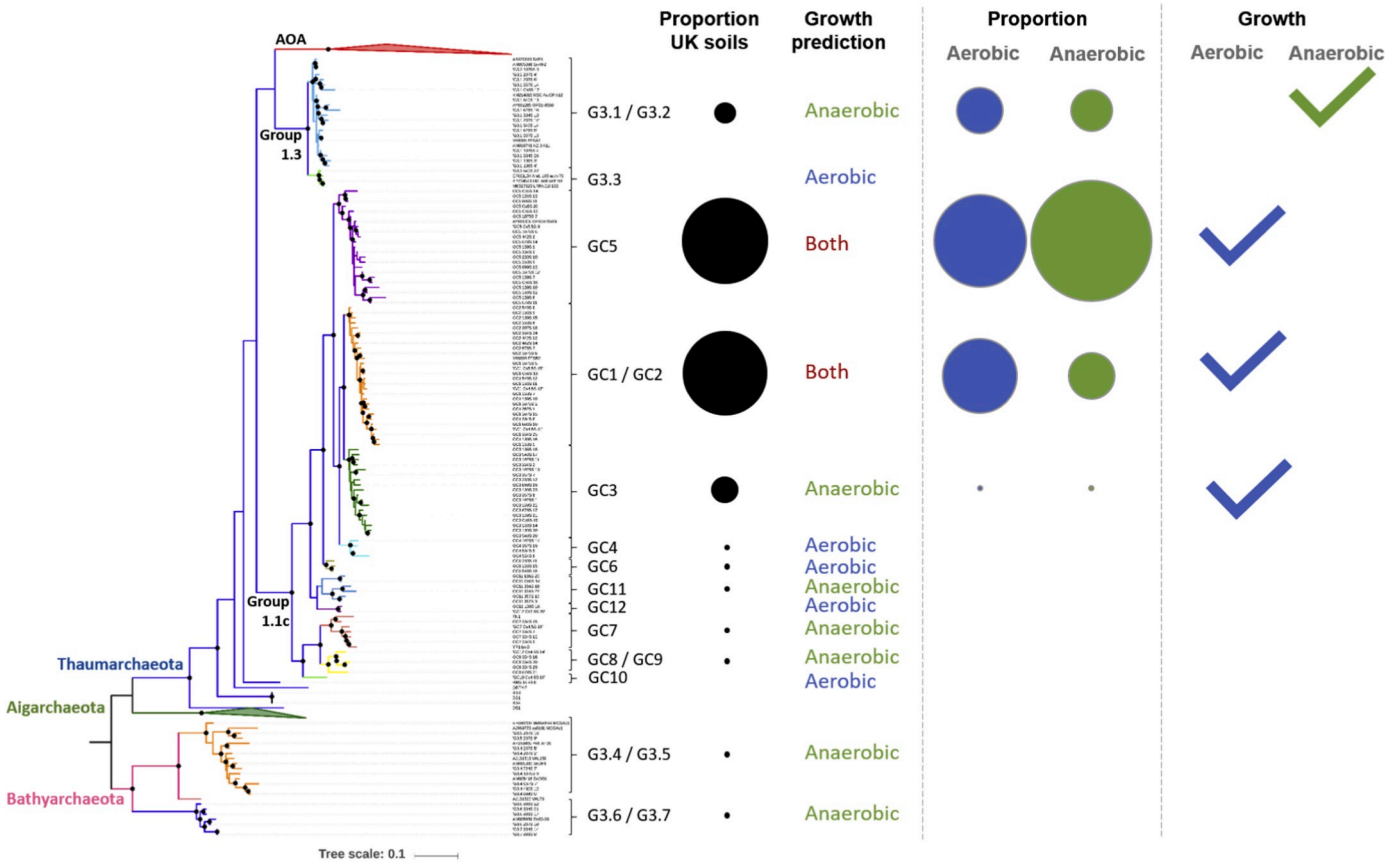

Fig. 1. A phylogenetic tree of mesophilic Group 1.1c and Group 1.3 Thaumarchaeota, based on previously published sequence diversity (Vico-Oton et al., 2016). The proportion of sequences in 48 UK soils is indicated by the size of the black circles alongside their predicted aerobic and/or anaerobic growth, based on the measured water content in the environments from which they originated (>70% was considered anoxic environment). The coloured circles indicate the proportion of the phylogenetic clusters in the oxic (blue) and anoxic (green) soils sampled in this study. Evidence obtained for aerobic and/or anaerobic growth of the different phylogenetic clusters investigated is indicated by ticks. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2. Materials and methods

2.1. Soil sampling and microcosm preparation

Five randomised soil cores were taken in May 2015 from an acid forest soil at the Fersit site, Scotland (56°52′01.8″N 4°41′46.1″W), previously described by Weber et al. (2015). The soil is classified as peaty gleysol, according to National Soil Map of Scotland (http://map.environment.gov.scot/Soil_maps,Fersit/Fersaid,19.12.2019). Oxic (20 cm depth) and anoxic (70–100 cm depth, below the water table level) soils were sampled and the anoxic soil cores were stored in an airtight glass jar prior to analysis, after depletion of residual oxygen using anaerobic atmosphere generation bags (Thermo, UK). Measurement of the oxygen content was not taken directly in the field due to limitation of such approach, so the soil status as oxic or anoxic was based on the soil type and the depth of the sampling.

Microcosms containing sieved (3.3-mm mesh size) oxic or anoxic soil were each incubated under oxic or anoxic conditions and headspace oxygen concentration was monitored weekly during incubation using an optical oxygen meter (FireStingO2, Pyro Science, Germany). Microcosms contained 50 g soil supplemented with 0.01% (v/v) acetylene, to inhibit growth and activity of ammonia oxidisers, and bottles were sealed by a crimped butyl rubber septum. Soils incubated under anoxic conditions (<0.05% oxygen) were handled in an anaerobic bag under a CO_2 atmosphere, while bottles containing soils incubated under oxic conditions (~21% oxygen) were aerated every 3 days. Addition of CO_2 (instead of N_2 or He) was used due to the large quantities required and to favour growth of some anaerobic microorganisms (Reilly, 1980). Microcosms were incubated for 45 days at 25 °C with weekly, non-destructive sampling of 3 g of soil (pooling three cores taken from each individual microcosm). Soil samples were then stored at −80 °C until further analysis.

2.2. DNA extraction and quantitative PCR

DNA was extracted from 0.5 g soil as described previously (Griffiths et al., 2000) and cleaned twice with AMPure® XP magnetic beads (Invitrogen, USA) (1.8:1 bead:sample ratio (v/v)), according to the manufacturer's instructions. Growth of Group 1.1c Thaumarchaeota in soil was assessed by quantitative PCR (qPCR) estimation of Group 1.1c 16S rRNA gene abundance using SsoAdvanced™ Universal SYBR® Green Supermix (BioRad, UK) with 318F (Weber et al., 2015) and 385R (Lehtovirta et al., 2009) primers, which partially cover the global Group 1.1c 16S rRNA phylogenetic diversity (i.e. thaumarchaeotal clusters GC1 to GC5 (Vico-Oton et al., 2016)). The assay was performed in a 20-µl reaction mix containing 10 µl of mastermix, 500 nM of each primer, 5 µl H_2O and 3 µl sample diluted to 2 ng DNA μl^{-1}. Cycling conditions were one cycle of 98 °C for 2 min, 35 cycles of 98 °C for 10 s, annealing and extension at 60 °C for 15 s followed by measurement of fluorescence and 1 cycle of 72 °C for 5 min. Finally, melting curve measurement was performed at 0.2 °C intervals for 20 min, from 60 °C to 95 °C. The assay had 98% efficiency and an R^2 value of 0.999.

2.3. High throughput sequencing

Changes in thaumarchaeotal communities during incubation of oxic and anoxic microcosms were assessed by MiSeq sequencing of Thaumarchaeota-specific 16S rRNA genes following PCR amplification using primers A109f (Großkopf et al., 1998) and 752r (reverse complement of 771f) (Ochsenreiter et al., 2003) containing additional specific MiSeq-tailed sequences, following manufacturer's recommendations. All amplifications were performed in a 25-µl reaction using the KAPA HiFi HotStart ReadyMix (Kapa Biosystems) with 0.4 µM of each primer and 40–60 ng template. Thermal cycling conditions were 95 °C for 3 min followed by 35 cycles of 98 °C for 20 s, 58 °C for 15 s, 72 °C for 20 s, followed by 72 °C for 5 min. The PCR-amplified sequences were cleaned using AMPure® XP beads and PCR-indexing was performed using the Nextera XT Index Kit according to the manufacturer's protocol. Library quantification, normalisation and pooling of 140 samples were performed prior to paired-end V3 MiSeq sequencing using 25% of PhiX spiking, enabling production of 2 × 300-bp reads.

Sequencing generated non-overlapping read lengths, necessitating the use of an assembly strategy adapted from the archaeal *amoA* gene (Aigle et al., 2019), termed the 'gap' pipeline. In brief, the paired-end raw reads were trimmed (-q 15) using Trim Galore (V0.4.5 (Krueger, 2015),) and filtered using the filterAndTrim command from the DADA2 package (maxEE = c(2,2), maxN = 0) (Callahan et al., 2016). Minimal size selection and read truncation (truncLen = c(200,200), minLen = 200) were applied and the reverse reads were further reverse-complemented and concatenated with the forward reads. The assembled reads were dereplicated at 100% sequence identity using usearch (-cluster_fast -centroids -sizeout) and chimeras and singletons were removed using unoise3 (Edgar, 2016). Finally, cleaned sequences were affiliated to different phylogenetic clusters using a thaumarchaeotal 16S rRNA gene sequence database (Vico-Oton et al., 2016).

2.4. Phylogenetic analysis

Phylogenetic analysis was performed on 169 sequences (average length of 700 bp), comprising published 16S rRNA gene sequences from genomes of deeply-rooted Thaumarchaeota and several Aigarchaeota and Bathyarchaeota retrieved from public databases and representative terrestrial sequences (recovered using primers 109f-752r) (Vico-Oton et al., 2016). Sequences were aligned with mafft using the L–INS–I method (Katoh et al., 2005) and trimmed (-automated1) using trimal (Capella-Gutierrez et al., 2009) and a phylogenetic tree was built using iqtree (Nguyen et al., 2015) with 1000 ultrafast bootstraps (-alrt 1000) (Guindon et al., 2010). Cluster affiliation was performed as described in Vico-Oton et al. (2016).

2.5. Statistical analysis

The effect of metabolism (aerobic vs anaerobic) and time on non-AOA thaumarchaeotal 16S rRNA gene abundance was determined by comparison of means from triplicate microcosms using two-way ANOVA in SigmaPlot (Systat Software, Inc., UK) and means of each condition were compared using the Fisher's least significant difference *post-hoc* test. Absolute abundance of the specific Group 1.1c thaumarchaeotal community was calculated as the specific Group 1.1c thaumarchaeotal qPCR values multiplied by the corresponding relative abundance obtained from the MiSeq sequencing. Changes in the absolute specific Group 1.1c abundance were determined by comparison of means from triplicate samples using one-way ANOVA with time as a factor and significance were assessed at $p < 0.05$ in all statistical analysis.

3. Results

3.1. Temporal changes in non-AOA thaumarchaeota community composition

This study focused on the most abundant Group 1.1c and Group 1.3 Thaumarchaeota in mesophilic terrestrial ecosystems, within the large diversity of non-AOA Thaumarchaeota (Hua et al., 2018; Ren et al., 2019). Temporal changes and growth of these lineages were assessed in two soils (originating from oxic or anoxic layers) incubated under oxic and anoxic conditions, using MiSeq sequencing and qPCR quantification of the thaumarchaeotal 16S rRNA genes. A phylogenetic tree reconstructed from 16S rRNA gene sequences of thaumarchaeotal mesophilic clades (Fig. 1) provided strong phylogenetic support for the majority of the nodes, and phylogenetic affiliations were broadly congruent with those previously described (Vico-Oton et al., 2016), with the exception of clades G3.4 to G3.7, which grouped with Bathyarchaeota rather than

Thaumarchaeota. Nineteen phylogenetic clusters were detected in all soil samples (Table 1), which were dominated by clusters previously considered to be most abundant globally (Group 1.3 G3.1/G3.2, Group 1.1c GC5, GC1/GC2 and GC3).

Initial community composition of the two soils differed in the initial abundance of Group 1.1c GC2, which was higher in oxic soil, and Group 1.1c GC5, which was higher in anoxic soil (Fig. 2, Table 1). Temporal changes in community composition were determined by analysis of relative abundance of each cluster after incubation for 0, 24 and 40 days (Fig. 2, Table 1). The relative abundance of Groups 1.1c GC1 and 1.1c GC3 increased significantly ($p = 0.032$ and $p = 0.003$, respectively) in oxic soil incubated under oxic conditions, while relative abundance of Group 1.1c GC2 increased significantly ($p < 0.001$) in both soils incubated under oxic conditions but remained constant under anoxic conditions. The relative abundance of Group 1.1c GC5 decreased significantly in the anoxic soil incubated under both anoxic ($p = 0.043$) and oxic conditions ($p = 0.08$). The relative abundance of Group 1.3 contrasted with that of Group 1.1c, with a significant decrease ($p < 0.05$) in oxic soil under oxic incubation and a significant increase ($p < 0.001$) during anoxic incubation of both soils (Fig. 2A).

3.2. Growth of non-AOA thaumarchaeota under oxic and anoxic conditions

Growth of each cluster was assessed as the product of cluster relative abundance and 'total' Group 1.1c thaumarchaeotal abundance, quantified by qPCR (Fig. 3). The primers used targeted thaumarchaeotal Group 1.1c clusters GC1 to GC5, but the existence of several primer mismatches prevented successful amplification of the less abundant thaumarchaeotal Group 1.1c clusters GC6 to GC12, and clusters belonging to the 1.3 Thaumarchaeota. Group 1.1c thaumarchaeotal gene abundance was higher in the oxic soil (3.22×10^6 g^{-1} dry soil) than in the anoxic soil (1.79×10^6 g^{-1} dry soil) (Fig. 3) but did not change significantly during incubation under oxic conditions. Under anoxic conditions, abundance decreased significantly ($p < 0.001$) by approximately 50% during the first 7 days of incubation, with a further smaller decrease between 7 and 24 days, to 27% of the original abundance. Oxic incubation of anoxic soil resulted in significant growth of Group 1.1c Thaumarchaeota with a 62% increase in gene abundance during the first 7 days ($p < 0.001$). Group 1.1c thaumarchaeotal growth was further observed during incubation for 42 days, but the final gene abundance was not significantly different from the initial ($p = 0.803$) or final ($p = 0.280$) abundances of the oxic soil under oxic incubation (Fig. 3). Group 1.1c thaumarchaeotal 16S rRNA gene abundance in the anoxic soil did not change significantly during the first 14 days of anoxic incubation, but then decreased significantly ($p = 0.010$). However, under those anoxic conditions, the final Group 1.1c thaumarchaeotal abundance in the anoxic soil remained significantly higher ($p < 0.001$) than that in the oxic soil (Fig. 3).

The absolute gene abundance for each Group 1.1c Thaumarchaeota phylotype was calculated by multiplying the total Group 1.1c thaumarchaeotal 16S rRNA gene abundance by the relative abundance of corresponding OTUs (Fig. 2B). Thaumarchaeotal Group 1.1c GC1, GC2 and GC5 grew in the anoxic soil under oxic incubation, but none of the thaumarchaeotal Group 1.1c clusters investigated grew under anoxic incubation (Fig. 2B).

4. Discussion

Deeply-rooted Thaumarchaeota (including Group 1.1c Thaumarchaeota) have previously been detected in several environments using molecular techniques, including boreal pine forest soils (Bomberg et al., 2011; Isoda et al., 2017), Scottish forest soils (Weber et al., 2015-), pHlimited cave systems (Barton et al., 2014), boreal fen and bog soils (Lin et al., 2014; Lin et al., 2015), anoxic soils (Lin et al., 2014) and B and C horizons of grassland and forest soils (Hansel et al., 2008; Lu et al., 2017). These organisms often represent a large proportion of the Thaumarchaeota community and represented >96% and >98% of all Thaumarchaeota in oxic and anoxic Scottish pine forest soil studied here (Table 1). While many studies report contrasting dynamics of deeply-rooted and ammonia-oxidising Thaumarchaeota, abundances of both groups are high in some soils. For example, Yarwood et al. (2010) reported high abundances of Group 1.1c and Group 1.1a-associated Thaumarchaeota representing both more than 15% of Archaea. This suggests that contrasting abundances may reflect niche specialisation rather than antagonistic interactions.

Table 1
Mean relative abundance (based on three replicates) of all thaumarchaeotal phylogenetic clusters retrieved after incubation of oxic and anoxic soils for 0, 28 and 42 days under oxic and anoxic conditions.

			Group 1.3 Thaumarchaeota				Group 1.1c Thaumarchaeota												AOA		
Soil	Incubation	Time (days)	G3.1	G3.2	G3.3	G3.4	GC1	GC2	GC3	GC4	GC5	GC6	GC7	GC8	GC9	GC10	GC11	GC12	P	R	A
Oxic	Oxic	0	20.6	0.0	0.0	0.0	8.8	24.9	2.1	0.8	39.0	0.0	0.3	0.0	0.1	0.0	0.1	0.1	2.3	0.7	0.0
Oxic	Oxic	28	18.2	0.0	0.0	0.0	9.6	26.6	3.2	0.5	40.5	0.0	0.2	0.0	0.1	0.0	0.1	0.0	0.9	0.2	0.0
Oxic	Oxic	42	16.9	0.0	0.0	0.0	10.0	28.2	3.6	0.5	39.3	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.9	0.0	0.1
Oxic	Anoxic	0	20.6	0.0	0.0	0.0	8.8	24.9	2.1	0.8	39.0	0.0	0.3	0.0	0.1	0.0	0.1	0.1	2.3	0.7	0.0
Oxic	Anoxic	28	31.3	0.1	0.0	0.2	5.6	24.3	2.1	1.4	34.3	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.3	0.1	0.1
Oxic	Anoxic	42	27.2	0.0	0.0	0.0	6.4	24.6	2.2	0.8	37.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.1	0.1
Anoxic	Oxic	0	18.2	0.1	0.0	0.0	10.3	10.5	2.2	0.6	51.2	0.0	3.2	0.3	0.0	0.0	0.6	0.8	1.2	0.7	0.0
Anoxic	Oxic	28	19.4	0.1	0.0	0.0	11.5	13.7	2.2	0.5	48.9	0.0	1.0	0.2	0.0	0.0	0.4	1.0	0.5	0.3	0.0
Anoxic	Oxic	42	18.0	0.1	0.0	0.0	11.1	16.3	2.5	0.5	47.8	0.0	1.5	0.3	0.0	0.0	0.5	0.8	0.4	0.4	0.0
Anoxic	Anoxic	0	18.2	0.1	0.0	0.0	10.3	10.5	2.2	0.6	51.2	0.0	3.2	0.3	0.0	0.0	0.6	0.8	1.2	0.7	0.0
Anoxic	Anoxic	28	21.3	0.1	0.0	0.0	10.8	13.9	1.9	0.6	48.3	0.1	1.0	0.2	0.0	0.0	0.4	0.7	0.4	0.3	0.1
Anoxic	Anoxic	42	26.8	0.1	0.0	0.0	9.4	13.6	1.6	0.6	44.6	0.0	1.3	0.2	0.0	0.0	0.3	0.7	0.4	0.3	0.0

Relative abundance
0.0 51.2

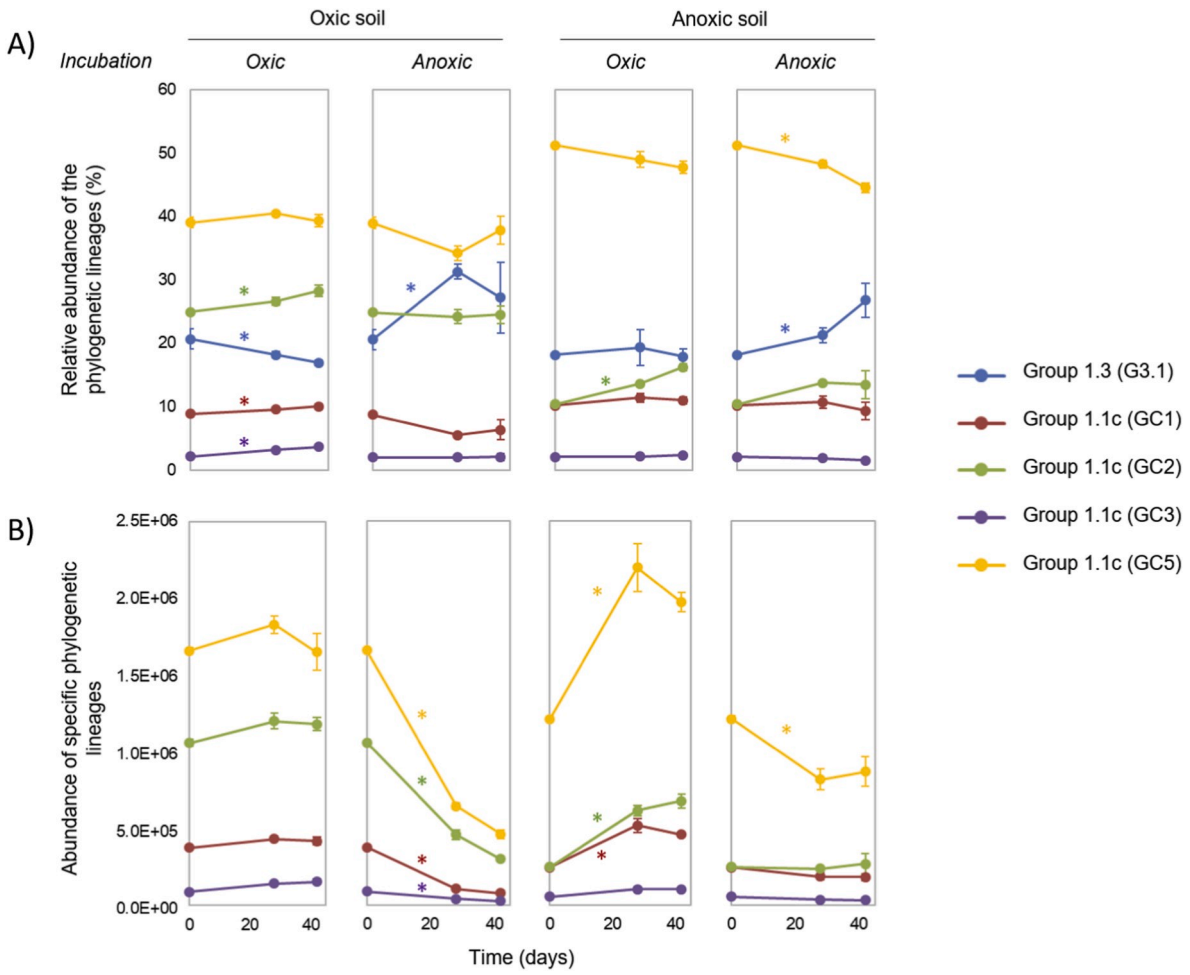

Fig. 2. Mesophilic thaumarchaeotal Group 1.1c and Group 1.3 community analysis within oxic and anoxic soils incubated under oxic or anoxic conditions for 42 days. A) Relative abundance of these phylogenetic groups (based on MiSeq sequencing using universal thaumarchaeotal primers). B) Absolute abundance of the five Group 1.1c phylogenetic clusters, calculated as the product of Group 1.1c 16S rRNA gene abundance (obtained by qPCR) and relative abundance of each phylogenetic cluster (obtained by MiSeq sequencing). Data are presented as mean and standard error of triplicate microcosms for each treatment. A star above each line represents a significant temporal difference in abundance ($p < 0.05$), based on a one-way ANOVA.

The main aim of this study was to test the oxygen preference of deeply-rooted, non-ammonia oxidising Thaumarchaeota, given speculation regarding their oxygen requirements (see Vico-Oton et al., 2016) arising, for example, from evidence for aerobic growth of mesophilic Group 1.1c Thaumarchaeota in soil (Weber et al., 2015), anaerobic growth of the cultivated representative of deeply-rooted Thaumarchaeota, isolated from an hyperthermophilic anoxic environment (Kato et al., 2019) and potential aerobic and anaerobic metabolism pathways indicated by a limited number of metagenome-assembled genomes (Beam et al., 2014; Lin et al., 2015; Hua et al., 2018). This study focused on mesophilic deeply-rooted Thaumarchaeota (including Group 1.1c and Group 1.3), with predictions of aerobic or anaerobic growth based on environmental metadata associated with abundant thaumarchaeotal 16S rRNA gene phylogenetic clusters (Vico Oton et al., 2016). While we focused our study on oxygen specialisation, other edaphic factors, e.g. sulphur, iron or fatty-acid concentration (Kato et al., 2019; Lin et al., 2015), may influence their *in-situ* growth more than oxygen.

Soil microcosm incubations provided evidence for aerobic growth of the three most abundant mesophilic Group 1.1c Thaumarchaeota clusters and anaerobic growth of the most abundant mesophilic Group 1.3 Thaumarchaeota (Fig. 2). This is consistent with aerobic growth of Group 1.1c Thaumarchaeota in a free-draining mineral podzol soil type (Weber et al., 2015), but not with predictions of anaerobic growth. Detection of anaerobic growth, previously reported in deep bog and fen soils (Lin et al., 2014, 2015), may require sampling of soil from greater depth (>50 cm from surface) and/or use of primers with broader coverage of Group 1.1c Thaumarchaeota diversity, as used in these studies. The lack of anaerobic growth was surprising, given the frequency of anoxic conditions in this soil due to high water table (Kleber et al., 2015), but oxygen may be supplied through pine roots (Roitto et al., 2019). Soil sampling was performed at the end of spring and the pine trees would have then entered their growing phase (Makkonen et al., 1997), and oxygen may have been released from pine roots providing favourable conditions for growth of aerobic thaumarchaeotal clusters, while the anaerobic thaumarchaeotal clusters may require a longer period of adaptation. Indeed, up to 100% oxygen saturation was reported in waterlogged soils when pine trees were in their growth phase, with only 0–20% oxygen saturation during the dormancy period (Roitto et al., 2019).

Soils sampled above and below the water table were assumed to be oxic and anoxic, respectively, and showed differences in community structure and archaeal abundance (Figs. 2 and 3; Table 1). The difference in abundance of aerobic cluster Group 1.1c GC5 Thaumarchaeota probably contributed to the subsequent observed growth, since the more favourable oxic conditions resulted in a significant increase in measured abundance. However, the initial differences in the two sampled soils had no obvious relevance to the observed growth of the anaerobic Group 1.3 Thaumarchaeota.

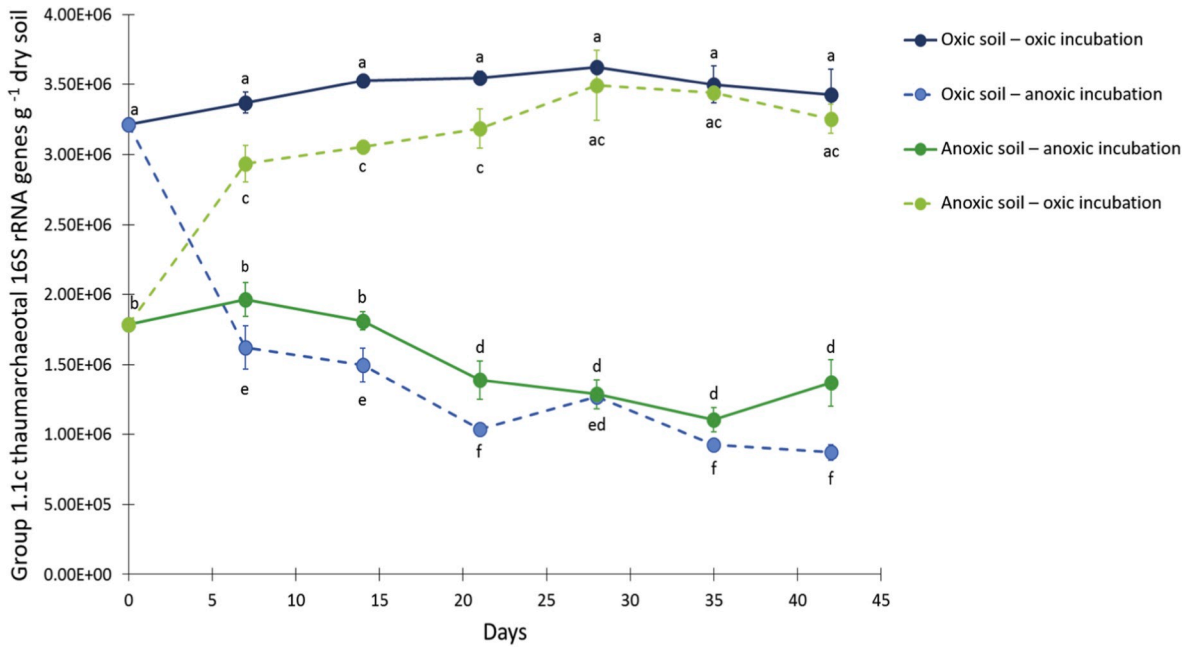

Fig. 3. Changes in abundance of Group 1.1c Thaumarchaeota during incubation of oxic (blue) and anoxic (green) soil for 42 days under initial (solid line) and contrasting oxygen (dashed line) concentrations. Data are presented as mean and standard error of triplicate microcosms for each treatment. Different letters above bars represent significant differences in abundance ($p < 0.05$), based on a two-way ANOVA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Growth of the mesophilic clusters of interest in oxic and anoxic soils was similar under anoxic conditions, with significant growth of Group 1.3 Thaumarchaeota in both soils (Fig. 2A), while growth of Group 1.1c Thaumarchaeotal clusters was only observed in the anoxic soil incubated under oxic conditions (Fig. 2B).

Final abundance of Group 1.1c Thaumarchaeota was similar in both oxic and anoxic soils incubated under oxic conditions (3.5×10^6 genes g^{-1} dry soil), which is in the same range as the abundance retrieved in previous Scottish pine forest soil studies (Lehtovirta et al., 2009; Weber et al., 2015). Similar abundance across different soil types and aboveground vegetation may suggest that Group 1.1c Thaumarchaeota populations have reached their carrying capacity under native conditions, which often occurs in soil microcosm studies after several weeks of incubation (Philippot et al., 2013; Tardy et al., 2014). Soil carrying capacity is defined as the maximum limit of the microbial community that the environment can support, and it is determined by the nutrient flux and soil physiochemical properties (McArthur, 2006). The most common nutrients that define the carrying capacity are organic carbon, nitrogen and phosphorous (Shackle et al., 2000; Remus-Emsermann et al., 2012; Verbruggen et al., 2012), but it is not known which factors limit Group 1.1c thaumarchaeotal growth. The abundance of Group 1.1c Thaumarchaeota in both soils was also similar (1.5×10^6 genes g^{-1} dry soil) following incubation under anoxic conditions and may represent dormant cells, surviving with energy expenditure being restricted to basic metabolism, until more favourable conditions permit growth (Hausmann et al., 2019). Incubation of anoxic soil under oxic conditions stimulated thaumarchaeotal growth of most phylotypes, indicating the ability of these microbes to persist under anoxic conditions. In addition to growth and dormancy, there was also evidence of cell death in some deeply-rooted mesophilic Thaumarchaeota clusters, especially of non-adapted populations under anoxic conditions. Interestingly, cell death stopped when population reached an abundance comparable to that of the native soil.

Future studies investigating the optimal growth conditions of mesophilic deeply-rooted Thaumarchaeota in soil are required in order to better understand conditions for their potential cultivation and consequently their environmental role. The current study focused on temporal changes in gene abundance, but temporal analysis of rRNA abundance in soils would be interesting (as rRNA is likely less persistent than DNA in soil) and it is likely that Thaumarchaeota would have a restricted transcriptional activity during dormancy (Lennon and Jones, 2011). Thaumarchaeotal cell death should also be analysed more thoroughly as DNA in soils can be degraded, adsorbed to soil particles or be assimilated by growing organisms. Several techniques would be particularly well-suited such as stable isotope probing to demonstrate growth (Papp et al., 2018; Zhao et al., 2020) and live-cell specific propidium monoazide-qPCR assays to demonstrate cell death (Soto-Muñoz et al., 2014).

In conclusion, this study suggests that mesophilic deeply-rooted Thaumarchaeota in this soil are composed of both aerobes and anaerobes, with a strong ability to persist under the alternate optimal environmental conditions. These Thaumarchaeota also encompass several subpopulations that have different dynamics, suggesting metabolic diversity within this group in relation to oxygen.

5. Availability of data and materials

Scripts used in this work can be found on GitHub (https://github.com/AigleAxel/amoA_MiSeq_sequencing/). Read data have been submitted to the Sequence Read Archive (SRA) under the accession number SUB7002919.

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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