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Intensive land use enhances soil ammonia-oxidising archaea at a continental scale

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ABSTRACT

Archaea are an important group of soil organisms that play key roles in carbon and nitrogen cycling, particularly in nitrification (ammonia oxidation) and methanogenesis. However, there are knowledge gaps regarding their importance in ecosystem processes relative to other microbial groups and how they may be impacted by land-use and environmental changes. Here, by carrying out a continental-scale sample collection and utilising archaea-specific primers for metabarcoding and shotgun metagenomics, we aimed to decipher the structure and function of archaeal communities across various land-use types in Europe. Metagenomic data reveal that land-use intensification increases the relative abundance of archaea, whereas bacteria and eukaryotes show no increase. Alongside this, ammonia oxidising archaea (AOA) increase as a proportion of the total metabarcoding reads, from 1 % of archaea in coniferous woodland to >90 % in croplands. Functional gene profiles reveal that land-use intensification shifts archaeal communities from adaptive metabolic pathways in forests to specialised, ammonia-oxidising microbes in fertiliser-enriched cropland soils. Our data suggest that land-use intensification may shift archaeal communities toward greater dependence on external nitrogen inputs, with potential consequences for soil fertility and greenhouse gas emissions.

1. Introduction

Climate and land-use changes pose a threat to biodiversity and ecosystem functioning. In particular, the adverse effect of land-use change (e.g., converting forests to agricultural areas) has been accelerated in recent years due to its interplay with climate change factors, resulting in biodiversity declines and ecosystem shifts at an unprecedented scale (Newbold et al., 2015). While several studies show that soil properties and climate factors are primary determinants of the structure of soil communities (Fierer, 2017; Bahram et al., 2018; Luan et al., 2023), the conversion of forests (hereafter woodlands) to agricultural lands (hereafter croplands) also contributes to shaping the diversity and composition of soil fauna, protists, bacteria and fungi (Tsiafouli et al., 2015; Köninger et al., 2023). To a large extent, these changes can be attributed to reduced carbon-to-nitrogen (C:N) ratios, higher pH, higher erosion and greater compaction along the land-use intensification

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gradient, while the relative effect of climate-related variables is negligible at the scale of Europe (Labouyrie et al., 2023). Land-use changes also have long-term consequences for C and N cycling and, thus, greenhouse gas (GHG) emissions, which are driven by soil microorganisms (Douglas et al., 2018; Tian et al., 2020). Land-use intensification is known to increase ammonium (NH_4^+) concentrations and soil pH, which can contribute to decreasing stocks of soil organic matter due to altered microbial C cycling efficiency (Malik et al., 2018). In this context, archaea are often overlooked despite being a ubiquitous and important player in N cycling with some unique biogeochemical capabilities.

Archaea play important roles in decomposing organic matter and cycling nutrients in terrestrial and aquatic ecosystems. Specifically, while bacteria are mostly related to decomposition, nitrification, and nitrogen fixation, and fungi to decomposition and symbiotic relationships with plants, archaea are responsible for key ecosystem functions such as nitrification and methanogenesis (Carey et al., 2016; Hink et al., 2018; Prosser et al., 2020; Bahram et al., 2022). Thus, changes in archaeal communities may alter fluxes of the powerful GHGs: methane (CH_4) and nitrous oxide (N_2O). Methanogenesis appears to occur only in archaea (Lyu et al., 2018). Ammonia-oxidising archaea (AOA) are one of the three major groups of microbes contributing to the first step in nitrification (i.e., ammonia oxidation), which is an aerobic process and both directly and indirectly drives N_2O production in soils (Prosser et al., 2020; Bahram et al., 2022). Preference for NH_4^+, along with differential preferences for nitrogen substrates like urea, has been suggested as a major determinant of the relative abundance of AOA, canonical ammonia-oxidising bacteria (AOB) and the comammox (Complete Ammonia Oxidation) bacterium *Nitrospira* (Xiao et al., 2020). AOA may be particularly successful because of their efficient autotrophic lifestyle and capacity for nitrification in various conditions and oxygen concentrations (Erguder et al., 2009; Stieglmeier et al., 2014). Besides direct N_2O production by AOA, AOB and comammox, aerobic nitrification can supply nitrate (NO_3^-) needed for anaerobic denitrification and further N_2O production. Furthermore, AOA have a high affinity for ammonia and a competitive advantage over heterotrophic bacteria and AOB under oligotrophic conditions (Martens-Habbena et al., 2009). In addition, temperature and pH optimum ranges of AOA have been shown to be broader than those of AOB (Hatzenpichler et al., 2008; Gubry-Rangin et al., 2011), particularly in acidic soils (Lehtovirta-Morley et al., 2011), which, together with their greater substrate affinity (Hink et al., 2018), imply niche differentiation between AOA and AOB across land-use intensification gradients. Organic matter and the C:N ratio have also been reported to influence the archaeal community composition (Oton et al., 2016).

Conversion of forests to croplands may affect soil microbial diversity through shifts in external nutrient inputs, which in turn modify soil properties like pH, nutrient stoichiometry, and soil organic carbon, but also soil oxygen level, to which archaea respond strongly due to their specific aerobic (e.g. nitrification) or anaerobic (e.g. methanogenesis) lifestyles (Bahram et al., 2022). Land-use change can also alter temporal and microscale spatial variations in soil oxygen levels through changes in soil structure, such as increased soil bulk density, reduced infiltration rates (van der Sande et al., 2022), potentially affecting soil archaeal communities. Yet, our understanding of archaeal community diversity and functional potential across different land-use types remains limited. While there have been previous studies linking archaeal diversity to the key environmental factors e.g. ammonia concentration, pH, land-use, organic matter and C:N ratio, there are few biogeography studies of archaeal community composition on continental scales (Bates et al., 2011; Starke et al., 2021). Numerous ecological surveys on archaea have been conducted on smaller scales (Tripathi et al., 2015; Karimi et al., 2018; Armbruster et al., 2021), but direct comparisons are usually complicated by different sampling strategies and procedures for sample preparation.

In this study, we examined the structure (16S rRNA gene metabarcoding) and potential gene functions (metagenomics) of archaeal communities across the major land-use types (cropland, grassland and woodland) on a continental scale (Fig. 1). We conducted an extensive field study and used highly standardized sample collection and processing methods in 881 study sites along a latitudinal gradient in Europe, within the framework of the soil module of the Land Use/Cover Area frame Survey (LUCAS – (Orgiazzi et al., 2018)). In particular, six vegetation cover types were sampled in semi-natural and highly managed areas, including coniferous and broadleaved forests, extensively and intensively managed grasslands, and permanent and non-permanent croplands (Orgiazzi et al., 2022). We determined both taxonomic diversity and composition (using newly sequenced PacBio-based long amplicons of 16S rRNA genes) and functional gene composition (shotgun metagenomics) for archaea, as well as vegetation cover (and associated land-use), soil physico-chemical properties, and climate variables. We also calculated the proportion of archaea to other organism groups in metagenomes based on rRNA gene relative abundances. We hypothesised that with increasing land-use intensity, the role of archaea in ecosystem functioning becomes more pronounced, as reflected in their greater relative metagenomic abundance. More specifically, we hypothesised that the functional gene diversity and, to a lesser extent, taxonomic diversity of archaea increase in response to land-use intensity due to increased nutrient availability.

2. Material and methods

Within the framework of the LUCAS Soil Biodiversity project (Orgiazzi et al., 2022), 881 composite soil samples (pools of five subsamples spanning a 4 × 4 plot, up to 20 cm depth) were collected in 2018 from the EU and UK. The sampling points were chosen to encompass a wide environmental gradient based on soil physico-chemical properties, topography, climate and land cover. For each location, 500 g of soil was collected and frozen at −20 °C within 48 h.

2.1. Soil chemical and physical properties

For each sample, using International Organization for Standardization (ISO) methods, soil properties were determined, including bulk

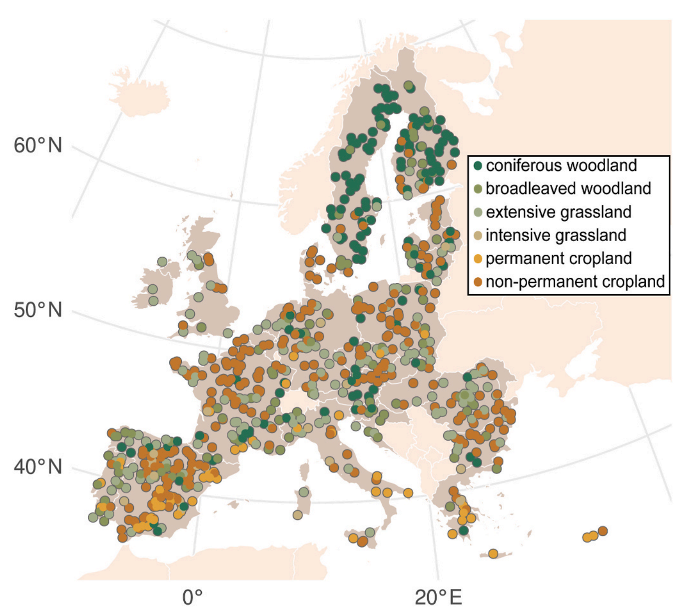

Fig. 1. Map showing sampling site locations. Colours indicate vegetation cover types as indicated in the legend. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

density (0–20 cm, $g.cm^{-3}$), clay and silt contents (%), coarse fragments (%), calcium (Ca) carbonate content ($g.kg^{-1}$), extractable potassium (K) content ($mg.kg^{-1}$), pH (in H_2O and CaCl), available phosphorus (P) content ($mg.kg^{-1}$), organic carbon (C; $g.kg^{-1}$), total nitrogen (N) content ($g.kg^{-1}$) and electrical conductivity (EC; $dS.m^{-1}$). Bulk density (BD, $g.cm^{-3}$) was determined following the ISO 11272:2017, based on the dry weight of soil in a given volume by accounting for both the solid part and the pore spaces of the soil (Panagos et al., 2024). For the first time, LUCAS included the analysis of almost 6000 samples of BD (Panagos et al., 2022). For more details, see Tables S1 and S2.

2.2. Molecular analysis

DNA was extracted in the molecular biology laboratory of the Mycology and Microbiology Center (University of Tartu) using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany) and quantified with the Qubit™ 1X dsDNA HS Assay Kit using a Qubit 3 fluorometer (Invitrogen, Carlsbad, USA). PCR amplifications were performed using the primers SSU1ArF (5′-TCCGGTTGATCCYGCBRG-3′) and SSU1000ArR (5′-GGCCATGCAMYWCCTCTC-3′) (Bahram et al., 2019). Each PCR was performed in two replicates, using 5 × HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia) in a total reaction volume of 25 μl. The archaeal DNA was amplified through 35 PCR cycles. In case of failed amplification, the procedure was adjusted by adding two or five PCR cycles, or the DNA was re-extracted and re-purified. Thermal cycling included an initial denaturation at 95 °C for 15 min; 25–40 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 55 °C, elongation for 1 min at 72 °C; final elongation at 72 °C for 10 min; and storage at 4 °C. The two replicates of each reaction were pooled and visualised on TBE 1 % agarose gel. DNA sequencing was performed using the PacBio Sequel II System (Pacific Biosciences, Palo Alto, USA).

For the functional metagenomic analysis, a subset of 630 samples was randomly selected from the total dataset, of which 601 were retained for this study, as 29 sites belonged to an under-represented land use type (e.g., bareland, shrubland and wetland). Library preparation and indexing of the pooled samples were performed using the Nextera XT DNA Library Prep Kit in combination with the Nextera XT Index kits v2 (Illumina, San Diego, USA). The metagenomes were sequenced using a shotgun approach, aiming for an expected depth of 10 million reads, on an Illumina NovaSeq platform at the University of Tartu with 2 × 150 bp paired-end mode.

3. Bioinformatics

3.1. Metabarcoding

Demultiplexing of sequences was performed using LIMA v.1.11.0 (Pacific Biosciences, Palo Alto, USA), with both dual barcodes required. Primer sequences were then trimmed using cutadapt v.3.0 (Martin, 2011). Sequences lacking primer matches were discarded. Additionally, sequences exhibiting more than two expected errors were excluded. Amplicon sequences were dereplicated and denoised using LotuS2 (v.2.25), using its default procedure for analysing PacBio data (Özkurt et al., 2022). *De novo* chimera detection and removal were carried out using the UCHIME3 algorithm (Edgar et al., 2011). Following initial processing, sequences were clustered using CD-HIT (Fu et al., 2012), based on 97 % similarity, to identify operational taxonomic units (OTUs) following (Grant et al., 2025). We also verified our OTU-based findings by analysing ASV-level data, using DADA2 implemented in the LotuS2 pipeline. We opted to use OTU in order not to lose rare taxa (Tedersoo et al., 2022). Taxonomic annotation of these OTUs was conducted against the KSGP database V1.0 (Grant et al., 2025). To mitigate the impact of potential tag-jump artefacts, the UNCROSS2 algorithm (Edgar, 2018) was applied within each sequencing run. Potential functional groups were associated with archaeal OTUs using the FAPROTAX functional trait database (Louca et al., 2016) based on OTU taxonomy. For this analysis of archaeal functional groups, we focused on AOA, due to their ecological relevance and dominance in our dataset.

3.2. Metagenomics

Quality control of metagenomics sequencing data was performed using MultiQC v.1.10 (Ewels et al., 2016). The preprocessing of reads, including quality filtering, adapter removal, and trimming of poly-G tails exceeding four bases, was conducted with fastp v.0.20.1 (Chen et al., 2018). During quality filtering, reads containing more than 20 % bases with Phred score <24 were excluded. Reads exhibiting three or more expected errors, indicating low quality, were also removed. Sequencing error correction was accomplished using the *clumpify* and *tadpole* functions from BBTools v.38.87 (Bushnell, 2014). Functional annotation of the quality-filtered reads was performed using eggNOG-mapper v.2.1.2 (Cantalapiedra et al., 2021) and the corresponding eggNOG orthology database v.5.0.2 (Huerta-Cepas et al., 2019). For homology search, we used DIAMOND v.2.0.10 (Buchfink et al., 2021) in blastx mode, following the methodology described by (Bahram et al., 2018). Next, to identify sequences originating from Archaea, we extracted all functionally annotated OGs assigned to the NCBI Taxonomy ID 2157 and its descendants. These OGs were grouped into broadly defined functional categories (Riley, 1993). To examine the relative proportion of rRNA genes of archaea, bacteria and eukaryotes within the communities, we utilized Metaxa2 version 2.2.3 (Bengtsson-Palme et al., 2015) to identify the taxonomy of both the small (SSU) and large (LSU) subunits of the rRNA genes present in the metagenomic datasets. We excluded rRNA from chloroplasts and mitochondria when calculating relative abundances.

3.3. Vegetation cover

The vegetation data were gathered through direct observations made by surveyors in the field. Each site’s vegetation was classified according to the EUROSTAT classification (https://ec.europa.eu/eurostat/documents/205002/8072634/LUCAS2018-C3-Classification.pdf). Vegetation was then grouped under larger vegetation cover types (e.g., non-permanent crops, permanent crops) following Labouyrie et al. (2023). Three LUCAS surveys have been conducted from 2009 to 2018 (every three years approximately, with the first survey: 2009–2012, the second survey: 2015, the third survey: 2018). Cropland, grassland, and forest were the three main land cover types chosen for reliable statistical studies. Nevertheless, each of these categories contained subcategories. In particular, croplands included permanent crops (such as fruit trees, olive groves, and vineyards) and non-permanent crops (e.g., cereals and legumes). Grasslands included intensive grasslands, which were defined as former croplands that had not been farmed for at least a year and had not been included in a crop rotation at the time of the study, or vast grasslands, i.e., permanent grasslands covered by communities of grassland and grass-like plants and forbs. Woodlands included broadleaved or coniferous forests. These categories represent a gradient of land-use intensity, from forests (least disturbed) to grasslands and croplands (most disturbed); for more details, see (Labouyrie et al., 2023).

3.4. Climate variables

Bioclimatic variables, including annual mean temperature, temperature seasonality, mean temperature of wettest quarter, maximum temperature in warmest month, mean diurnal range, isothermality, annual precipitation, and precipitation seasonality, were extracted from the CHELSA database v.1.2, derived from data covering the period from 1979 to 2013 (Karger et al., 2020) based on the geographic coordinates of the samples.

3.5. Statistical analyses

All statistical analyses were conducted using R v.4.2.1 (R Core Team, 2024). The data were rarefied to account for differences in sequencing depth across samples. The Shannon diversity index was calculated from the rarefied abundance matrices using the *vegan* package v.2.5–6 (Oksanen et al., 2007). PERMANOVA and pairwise PERMANOVA were performed to test OTU and OG composition discrimination across land-use types, using functions *adonis2* and *pairwiseAdonis* of *vegan* and *pairwiseAdonis* v.0.4.1 packages, respectively. For between-group comparisons, P-values were adjusted using the Bonferroni correction. We further visualised the taxonomic (OTU) and functional (orthologous gene; OG) composition of archaea using global nonmetric multidimensional scaling (GNMDS) in vegan based on the following options: two dimensions, initial configurations = 100, maximum iterations = 200, and minimum stress improvement in each iteration = 10^{-7}. To assess the concordance between the taxonomic and functional ordinations, we applied Procrustes analysis in *Vegan*. Multivariate analyses were performed using Bray-Curtis dissimilarity matrices. We used Spearman correlation analysis components to identify the archaeal taxonomic lineages related to GNMDS axes. In addition, *envfit* function of *vegan* was used to assess relationships between environmental variables and the GNMDS axes. Forward selection was used to preselect the important variables in explaining response variables, as implemented in *forward.sel* function of the *adespatial* v.0.3–23 package (Dray et al., 2023). Hierarchical partitioning was used to disentangle the individual contribution of environmental variables and land-use type on diversity measures using the *glmm.hp* package v.0.1–3 (Lai et al., 2022). For conducting between-group comparisons, we used estimated marginal means using the package *emmeans* v.1.9.0 (Lenth et al., 2024) for the univariate models, followed by multiple-testing correction using Tukey's method.

4. Results

4.1. Archaeal diversity and community composition

Altogether, a total of 881 samples were included in the 16S rRNA gene metabarcoding analysis using specific archaeal primers (Bahram et al., 2019). Of these, 808 samples with >1000 archaeal reads were retained for analysis, 743 samples originated from cropland, grassland, and woodland ecosystems and were included in the final dataset. After rarefying read abundances to 1000 reads per sample, the dataset included 20,939 archaeal OTUs at a 97 % sequence similarity cutoff. Metagenomic data (n = 601) revealed a similar proportion of Archaea (1.2 % ± 0.93; Mean ± SD) to the average 2 % reported across global soils (Bates et al., 2011). The dominant archaeal phyla were *Thermoproteota* (92.2 %), *Thermoplasmatota* (6.5 %), *Halobacteriota* (6.1 %), and *Nanoarchaeota* (6.1 %). At the order level, *Nitrososphaerales* (86.7 %), UBA184 (3.3 %), and B26-1 (1.2 %) were most abundant.

Archaeal OTU diversity differed significantly among land-use types (GLM with Gaussian distribution: Chi-square = 23.24, df = 5, $p < 0.001$), with higher diversity in intensive grassland and permanent cropland compared to broadleaved and coniferous woodland, and higher diversity in permanent cropland compared to extensive grassland (Fig. 2). Among abiotic variables, archaeal OTU diversity was related to soil pH ($r^2 = 0.008$, $p = 0.013$), soil P ($r^2 = 0.01$, $p = 0.001$), and precipitation of the driest month ($r^2 = 0.025$, $p < 0.001$). Together with land-use, these factors explained 6.6 % of the variation in archaeal diversity (Table S3). The small effect of climate aligns with the pattern reported for soil bacteria and fungi across Europe (Labouyrie et al., 2023; Netherway et al., 2024).

4.2. Community composition and environmental drivers

Land-use significantly affected OTU community composition (Permanova: $p = 0.001$), even after accounting for influential soil and climate variables (Table S4). Archaeal communities in croplands were more similar to grasslands ($r^2 = 0.021$, adjusted $p = 0.003$) than to woodlands ($r^2 = 0.147$, adjusted $p = 0.003$). The most distinct OTU compositions were between coniferous woodland and non-permanent cropland (Table S5). Soil pH was the strongest determinant of archaeal community composition (Fig. 3; Table S4), with large pH differences across land-use types, especially between cropland and woodland (Fig. 3d). *Thermoproteota* dominated most land-use types, except in intensive grassland and permanent cropland, where their relative abundance declined in favor of *Nanoarchaeota* and *Thermoplasmatota*

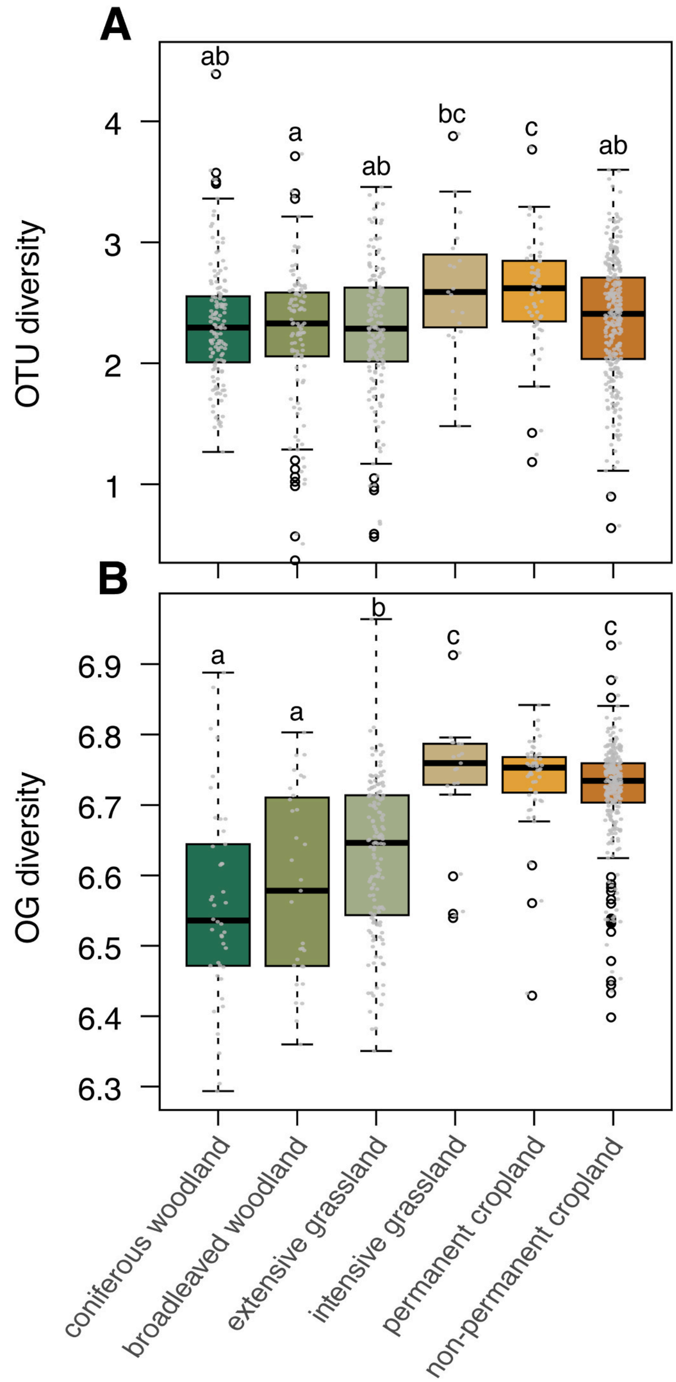

Fig. 2. Taxonomic and functional gene diversity of archaea across land-use types. Diversity was calculated based on the Shannon diversity index, with the data rarefied prior to the calculation. The significance of pairwise comparisons was adjusted based on the Tukey method. Abbreviations: OTU: operational taxonomic unit; OG: Orthologous genes.

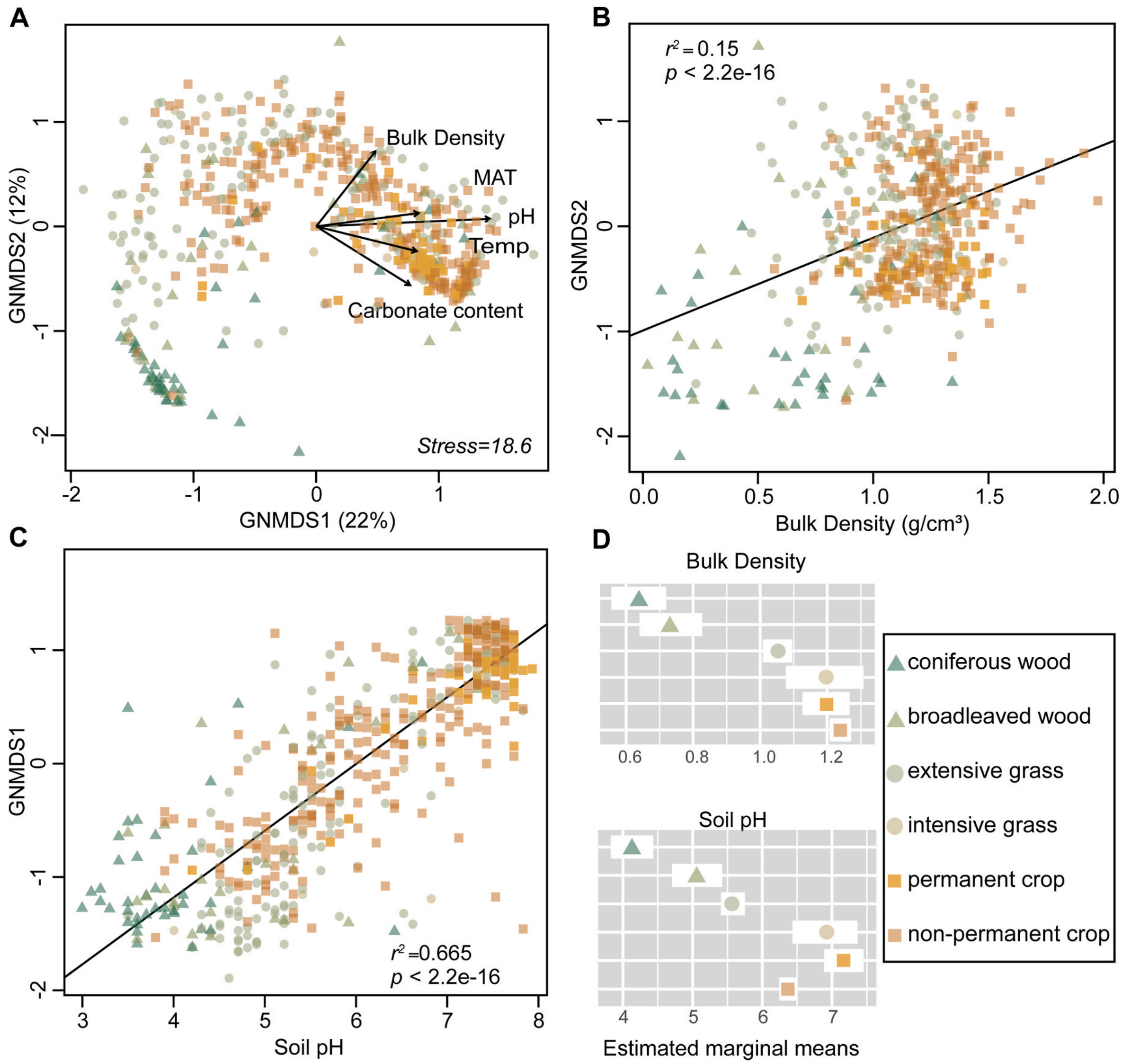

Fig. 3. A Global Nonmetric Multidimensional Scaling (GNMDS) plot showing variation in the archaeal OTU composition across different land-use types. Factors with $r^2 > 0.2$ determined using envfit function are shown. The ordination plot shows the percentage of explained variance in parentheses. The abundance of data was rarefied to the minimum shared number of reads (1000). **B, C,** The correlation of the first two GNMDS axes with a key associated environmental variable. **D,** The marginal effects plot, showing estimated marginal means (emmeans) of the key soil variables (i.e. soil pH and bulk density) associated with the first and second GNMDS axes for each land-use category (as shown in the legend). The 95 % confidence interval is represented by the shaded area surrounding the points. Abbreviations: MAT, mean annual temperature; Max Tem: Maximum temperature of warmest month.

(Fig. S1).

4.3. Functional group patterns and drivers

Land-use type also strongly influenced the relative abundance of archaeal functional groups. In particular, aerobic AOA (inferred from metabarcoding) increased with land-use intensification—from <1 % in woodlands to >90 % in croplands (Fig. 4; Table S6). Parametric forward selection showed AOA abundance was mainly related to soil pH ($t = 22.33$, $p < 0.001$), C:N ratio ($t = -6.74$, $p < 0.001$), bulk density (BD) ($t = 6.00$, $p < 0.001$), electrical conductivity (EC) ($t = -4.78$, $p < 0.001$), together explaining 67.0 % of the variation. A model including land-use had a significantly lower AIC score (9150 vs 10026; ANOVA: $F = 8.28$, $p < 0.001$). Hierarchical partitioning (Table S3) revealed land-use as the main factor ($r^2 = 0.13$, $p < 0.001$), followed by C:N ratio ($r^2 = 0.104$, $p < 0.001$), BD ($r^2 = 0.093$, $p < 0.001$), and soil pH ($r^2 = 0.087$, $p < 0.001$).

AOA abundance positively correlated with clay content ($r = 0.432$) and subsurface moisture ($r = 0.373$), and negatively with mean annual precipitation ($r = -0.205$), total nitrogen ($r = -0.204$), and organic carbon ($r = -0.368$). While the functional group of 31.2 % of OTUs remained unknown, methanogens comprised only 1.1 % of the archaeal community vs. 67.2 % for AOA, likely reflecting their anaerobic lifestyle and the non-anoxic conditions of study sites.

4.4. Metagenomics analysis of diversity and metabolic potential

Archaeal metabolic gene potential (based on metagenomic data, $N = 601$) was assessed by assigning sequences to orthologous groups (OGs). Archaeal OG diversity increased with land-use intensity ($p < 0.001$), highest in non-permanent croplands (Fig. 2). Hierarchical partitioning revealed that land-use type ($r^2 = 0.159$, $p < 0.001$), soil pH ($r^2 = 0.156$, $p < 0.001$), mean annual temperature (MAT) ($r^2 = 0.074$, $p < 0.001$), and soil P ($r^2 = 0.032$, $p < 0.001$), were significant determinants of archaeal OG diversity (Table S5). Analysis of metagenomic rRNA genes revealed that, the relative abundance of archaea significantly increases from

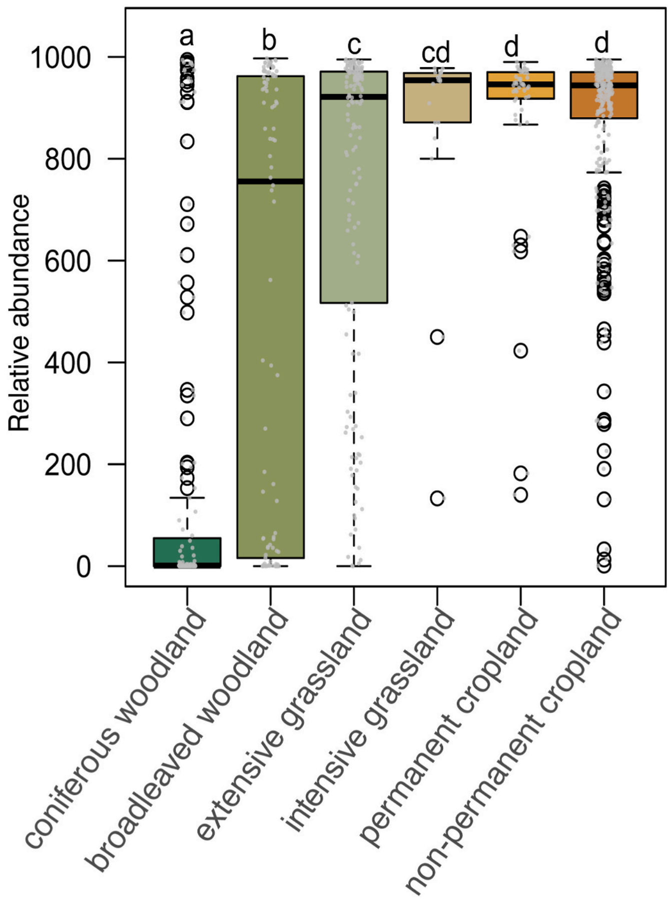

Fig. 4. The relative abundance of OTUs involved in aerobic ammonia oxidation across land-use types. The functional annotation was based on the *FAPROTAX* database. For aerobic ammonia oxidation, all comparisons were significant except for between extensive and intensive grasslands and between intensive grassland and croplands (See Table S3). Abbreviation: AOA: aerobic ammonia oxidising archaea.

woodlands to croplands, while bacteria and eukaryotes show no increase (Fig. 5).

4.5. Gene composition and key OTUs

The composition of OGs was significantly affected by land-use ($r^2 = 0.147$, $p = 0.001$). The strongest differences were between non-permanent cropland and coniferous woodland (Table S8; Figs. S2 and S3). Metabolic genes linked to the thaumarchaeotal carbon fixation pathway (e.g. 4-hydroxybutyrate-CoA ligase, acetyl-CoA/propionyl-CoA carboxylase, 3-hydroxypropionyl-CoA synthetase) increased with land-use intensity (Table S7), aligning with AOA abundance and their metabolic role in cropland soils. Three OTUs (OTU4, OTU1, OTU3) explained 83 % of the variation across the first NMDS axis. The relative abundance of Group 1.1c was primarily driven by land-use ($r^2 = 0.463$, $p < 0.001$), with soil pH as a secondary factor ($r^2 = 0.413$, $p < 0.001$). OTU and OG composition were strongly correlated (Mantel $r = 0.542$, $p = 0.001$), though correlations between taxonomic and functional diversity were significant only in croplands (Fig. 6, S5). While there is a statistically significant relationship between taxonomic and functional gene compositions across all land use types, the strength of this relationship varies considerably, with forested environments (coniferous and broadleaved woods) showing a tighter coupling compared to grasslands and croplands.

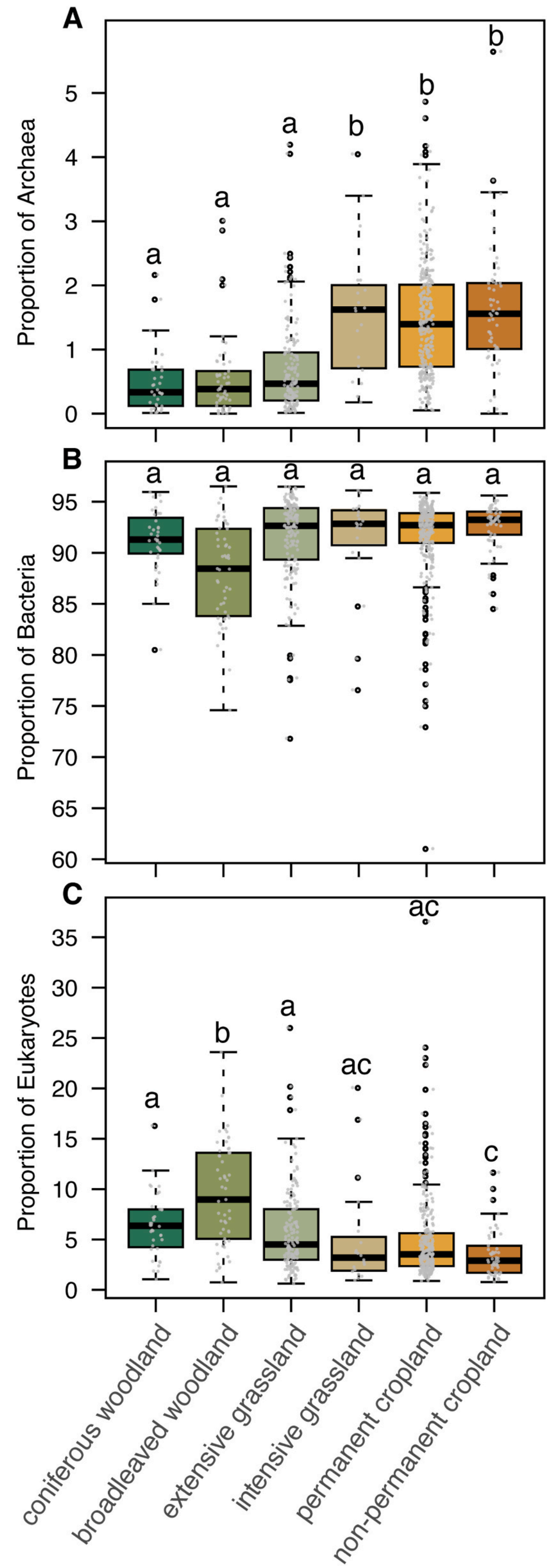

Fig. 5. Relative abundance of different kingdoms across different land-use types. The relative abundances were determined based on shotgun metagenomics rRNA genes.

5. Discussion

5.1. Land-use as a primary ecological filter

Our study demonstrates that land-use type is a key factor shaping soil

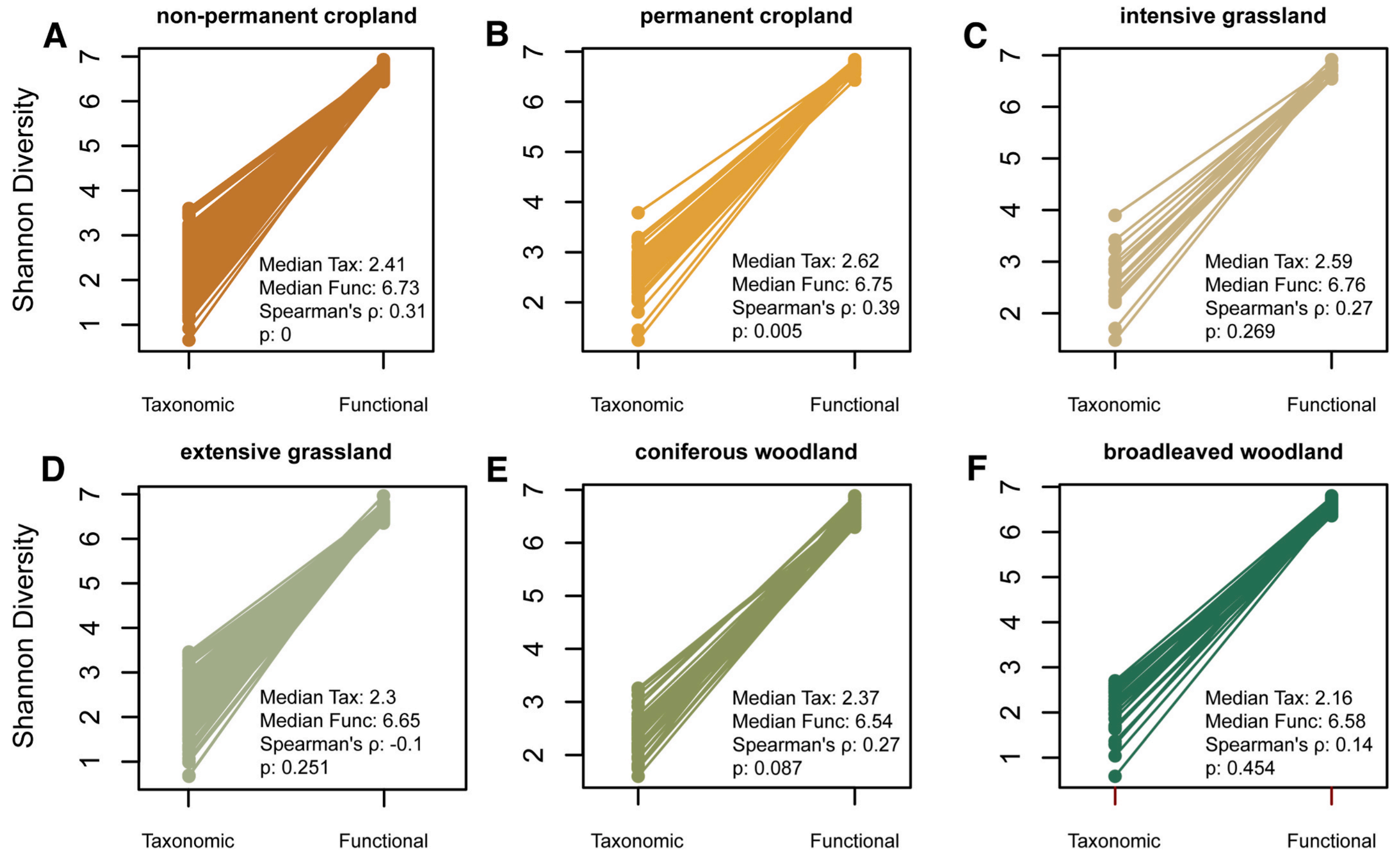

Fig. 6. Stronger relationships between functional and taxonomic diversity (Shannon diversity index) with increased land-use intensification. Spearman correlation analyses reveal that this relationship is statistically significant in non-permanent and permanent cropland samples, while it remains non-significant for other land-use types. The correlation coefficients and associated p-values are provided in the insets. Abbreviations: Tax: taxonomic diversity, estimated based on the Shannon diversity of operational taxonomic unit; Func: functional gene diversity, estimated based on the Shannon diversity of Orthologous genes.

archaeal community structure and function. Our results show that archaeal diversity, particularly AOA, increases in intensive grasslands and croplands compared to woodlands, supporting our hypothesis that land-use intensification shifts archaeal communities toward greater diversity and enhanced functional potential in nitrogen cycling. This aligns with earlier work on bacteria and fungi (Labouyrie et al., 2023), but here we show that archaea—often understudied in terrestrial systems—respond more strongly to land-use intensification. The higher dissimilarity between woodland and cropland archaeal communities (PERMANOVA: $r^2 = 0.165$, $p < 0.001$) reflects environmental filtering, likely driven by differences in nutrient availability. Furthermore, the stronger similarity between cropland and grassland archaeal communities, compared to woodland, may reflect shared disturbance histories and management practices, such as fertilization, tillage, or grazing. These likely homogenize soil conditions and select for archaeal taxa adapted to resource-constrained soil conditions. Similar increases in archaeal AOA relative abundance under fertilised or disturbed soils have been reported in previous studies (Leininger et al., 2006; Bates et al., 2011), while archaeal communities are dominated by non-AOA groups in forested or undisturbed soils (Tripathi et al., 2015), highlighting the importance of nutrient limitation in shaping archaeal communities.

Consistent with other studies on soil microbial biogeography (Lehtovirta et al., 2009a; Tripathi et al., 2015; Bahram et al., 2018; Karimi et al., 2018), we further found that soil pH is the primary environmental driver of archaeal community structure (Fig. 3c). Since liming raises soil pH, it is plausible that such agricultural practices favor AOA in croplands. Several mechanisms may explain why a more neutral pH promotes AOA abundance or activity. For instance, higher pH can increase the proportion of free ammonia relative to ammonium, enhancing substrate availability for AOA (Stempfhuber et al., 2015). Additionally, neutral pH conditions may reduce proton stress, lowering the energetic cost of maintaining intracellular pH homeostasis (Krulwich et al., 2011). Shifts in pH may also influence competitive interactions between AOA and bacterial ammonia oxidisers (AOB), but pH alone is unlikely to explain the results, given that AOA are well adapted to the acidic soils (Nicol et al., 2008). Together, these suggest that soil pH may not only structure archaeal communities but also modulate their functional roles in nitrogen cycling.

5.2. Functional specialisation and metabolic potential

A notable finding of our study is the increased functional gene diversity of archaea with land-use intensification, in contrast to trends observed in bacterial and eukaryotic microbial communities (Delgado-Baquerizo et al., 2016). Our metagenomic data show a greater relative abundance of genes involved in core metabolism, such as KEGG orthologs (KOs) related to acetyl-CoA/propionyl-CoA carboxylase and 3-hydroxypropionyl-CoA synthetase (Table S7). This implies that soil archaea not only dominate ammonia oxidation under high-intensity land use but also contribute to carbon cycling, particularly in nitrogen-rich croplands. Additionally, the relatively low abundance of methanogens (1.1 %) across all sites reflects the generally oxic and well-drained nature of our soils, suggesting that methanogenic pathways may be more relevant in specific microsites (e.g., root zones or compacted layers) or under different hydrological conditions. These results align with recent studies showing that AOA contribute to both nitrogen and carbon cycling in oxic soils, while methanogenic archaea are limited to anaerobic microsites or wetter environments (Kim et al., 2021; Wright and Lehtovirta-Morley, 2023).

5.3. AOA as functional keystones in croplands

The positive relationship of AOA with soil bulk density suggests that oxygen levels, which are expected to be lower at higher bulk densities, may not be the primary determinant of AOA or were at least not limiting in these samples. Conversely, their negative correlation with mean annual precipitation suggests that increased rainfall might limit AOA, possibly due to saturation or leaching effects. This is intriguing because clay particles, being negatively charged, bind ammonium, potentially making ammonia less available (Nieder et al., 2011), which may help AOA compete against AOB. In addition, AOA were negatively correlated with total nitrogen and organic carbon. These results suggest that AOA could play an underestimated role in ecosystems with low inputs of nitrogen and carbon, where ammonium availability is naturally limited by the mineralisation rate and microbial communities are more dependent on low-nutrient strategies. An alternative explanation is that soils with low organic matter (low C and N) exhibit reduced nitrogen immobilisation due to limited decomposition activity. Under such conditions, AOA may face less competition from heterotrophs for NH_4^+, potentially enhancing their ecological niche in nutrient-poor environments.

There is strong evidence that ammonia concentration is an important determinant of AOA abundance, as high levels of accessible ammonium tend to reduce AOA relative abundance, likely due to competition with nitrifying bacteria (Bates et al., 2011; Verhamme et al., 2011). How this affects the balance of AOA, AOB, and comammox *Nitrospira* warrants further investigation. This is especially relevant for land-use change, as forests converted to cropland are likely to retain high organic carbon content, typical of woodland soils. Overall, our data suggest that AOA diversity and abundance are mainly driven by soil pH and land-use intensification. Moreover, there may be indirect relationships between AOA and other microbial groups that affect carbon and nutrient cycling, which also deserve further study.

5.4. Key taxonomic groups and their ecology

The OTUs that most strongly correlated with NMDS axes of archaeal community composition all belonged to the class *Nitrososphaeria*. Notably, OTU4, part of Group 1.1c *Thaumarchaeota* (most similar to BOG-1369), is negatively impacted by land-use intensification and is primarily found in acidic soils (Fig. S4). It is more prevalent in coniferous forest soils than in deciduous forests or grasslands (Lehtovirta et al., 2009a; Bates et al., 2011; Weber et al., 2015). There was a strong negative correlation between Group 1.1c and ammonia monooxygenase, supporting reports that these archaea are unable to oxidize ammonia (Weber et al., 2015). Their high relative abundance may be due to aerobic growth conditions (Biggs-Weber et al., 2020) and non-reliance on ammonia oxidation, potentially benefiting from oxygen and organic compounds provided by tree roots and ectomycorrhizal fungi. Group 1.1c (f_UBA183 in Genome taxonomy database) has been proposed to conserve energy via beta-fatty acid oxidation based on metagenomic data from anoxic peat (Lin et al., 2015), and may also obtain nutrients from soil organic matter (Weber et al., 2015). In agreement with these findings, land-use type was the main factor affecting Group 1.1c abundance, with soil pH as a secondary factor. Group 1.1c was more represented under lower land-use intensity, yet no evidence supports its involvement in ammonia oxidation (Weber et al., 2015). Whether their higher abundance in forests is due to interactions with ectomycorrhizal fungi remains an open question. The consistent decline of Group 1.1c in croplands suggests that land-use intensification may lead to functional changes, especially for archaeal groups not directly involved in nitrogen cycling but possibly playing roles in organic matter turnover or symbiotic interactions in forest ecosystems. This is supported by Lehtovirta et al. (2009b), who found that Group 1.1c are dominant in acidic soils, including forest soils, implying specialised functions in organic matter turnover in undisturbed environments.

5.5. Greater functional redundancy in forest archaea

Our results support that while the taxonomic composition of archaea varies with land-use intensification, their functional potential is preserved, which is critical for maintaining ecosystem resilience (Tilman et al., 2006; Louca et al., 2016). In forests, particularly in coniferous forests, the Procrustes analysis shows the greatest decoupling between taxonomic and functional composition, i.e. even though taxonomic composition changes, the functional gene composition remains stable (Fig. S5). By contrast, in cropland, we found a tighter correlation between functional and taxonomic diversity, implying that functional resilience provided by functional diversity may be reduced (Fig. 6). In croplands, archaea may evolve more specialised functions to adapt to high nutrient availability and sudden management-related stresses, compared to more stable conditions and greater organic matter availability in forest soils. These data suggest that loss of taxonomic diversity could more immediately impair functional resilience in croplands compared to forests. Indeed, high microbial diversity with overlapping functions has been shown to be critical for sustaining ecosystem functionality in terrestrial systems (Delgado-Baquerizo et al., 2016), and loss of soil biodiversity—hence the redundancy of key functional traits—can lead to declines in ecosystem performance (Wagg et al., 2014). Our findings suggest that land-use intensification may reduce the redundancy of key functional traits, ultimately negatively affecting nutrient cycling and carbon storage.

Our functional gene data (Fig. S2; Table S7) further suggest that, despite a lower OG diversity, forest archaeal communities may be better adapted to natural soil fluctuations by maintaining a broad range of metabolic pathways for organic nutrient utilisation, as reflected in increased relative abundance of OGs related to ABC transporters, amino acid transporters, oxidoreductases, dehydrogenases, and phosphorylases as well as cytochrome P450 (Thomson et al., 2015; Armbruster et al., 2021; Huang et al., 2021). In contrast, cropland archaeal communities appear more adapted for fertiliser-enriched soils where consistent nutrient availability reduces the need for such metabolic versatility. This functional shift, favouring ammonia oxidation over complex organic nitrogen transformations under land-use intensification, could render intensively managed agricultural systems increasingly dependent on external nitrogen inputs, potentially impacting soil fertility and greenhouse gas emissions.

6. Conclusions

Our study suggests that land-use intensification significantly increases the relative abundance of archaea in the soil, a pattern not observed in soil bacteria and eukaryotes. The increase in archaeal relative abundance is especially notable among AOA, suggesting that agricultural intensification promotes nitrifying archaea, with potential consequences for N_2O emission (Bahram et al., 2022). Unlike bacteria, AOA may thrive in the altered soil chemistry, reinforcing their important role in nitrogen cycling under agricultural intensification (Hink et al., 2018; Huang et al., 2021). This corroborates a prior study highlighting a more pronounced role of AOA in nitrogen cycling in highly fertile agricultural soils compared to plant-covered or fallow lands (Huang et al., 2021). The autotrophic lifestyle and the high substrate affinity of AOA (Jung et al., 2022) may underlie the substantial role of this group in carbon and nutrient cycling in agricultural soils. However, their ecological advantages over AOB remain elusive (Walker et al., 2010). Moreover, the shift toward a AOA-dominated community in cropland soils implies that soil archaea may be less resilient to environmental disturbances, underscoring the need for further investigation into the responses of archaea under land-use intensification.

CRediT authorship contribution statement

M. Bahram: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **L. Lehtovirta-Morley:** Writing – review & editing. **V. Mikryukov:** Writing – review & editing, Data curation. **T.R. Sveen:** Writing – review & editing, Data curation. **A. Grant:** Writing – review & editing, Data curation. **M. Pent:** Investigation, Formal analysis, Data curation. **F. Hildebrand:** Methodology. **M. Labouyrie:** Writing – review & editing, Data curation. **J. Köninger:** Writing – review & editing, Data curation. **L. Tedersoo:** Writing – review & editing, Methodology, Formal analysis. **A. Jones:** Investigation, Funding acquisition. **P. Panagos:** Writing – review & editing, Investigation, Funding acquisition. **A. Orgiazzi:** Writing – review & editing, Investigation, Funding acquisition, Data curation, Conceptualization.

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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.2025.110024.

Data availability

Archaeal 16S rRNA gene sequences and shotgun metagenomics sequence data have been deposited in the Sequence Read Archive (SRA) database under BioProject ID PRJNA1118194 and PRJNA1032917, respectively. Metadata for soil properties measured in LUCAS 2018 survey are available on the European Soil Data Centre (ESDAC; https://esdac.jrc.ec.europa.eu/content/soil-biodiversity-dna-eukaryotes).

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