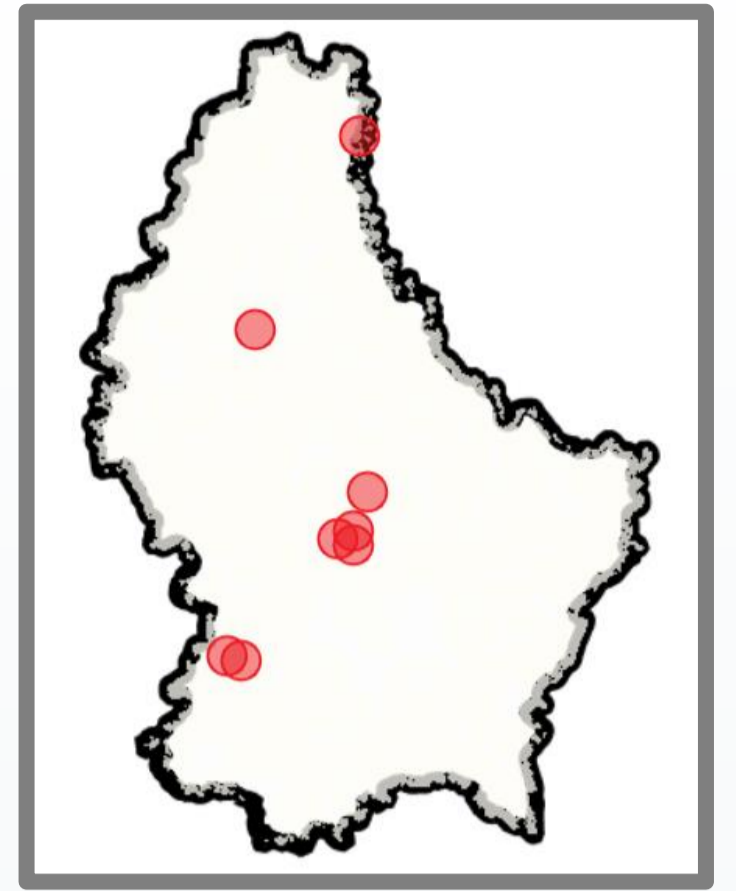


# Soil microbial biodiversity in Luxembourg

Kate M. Buckeridge, Xavier Goux, Malte Herold, Magdalena Calusinska  
Luxembourg Institute of Science and Technology

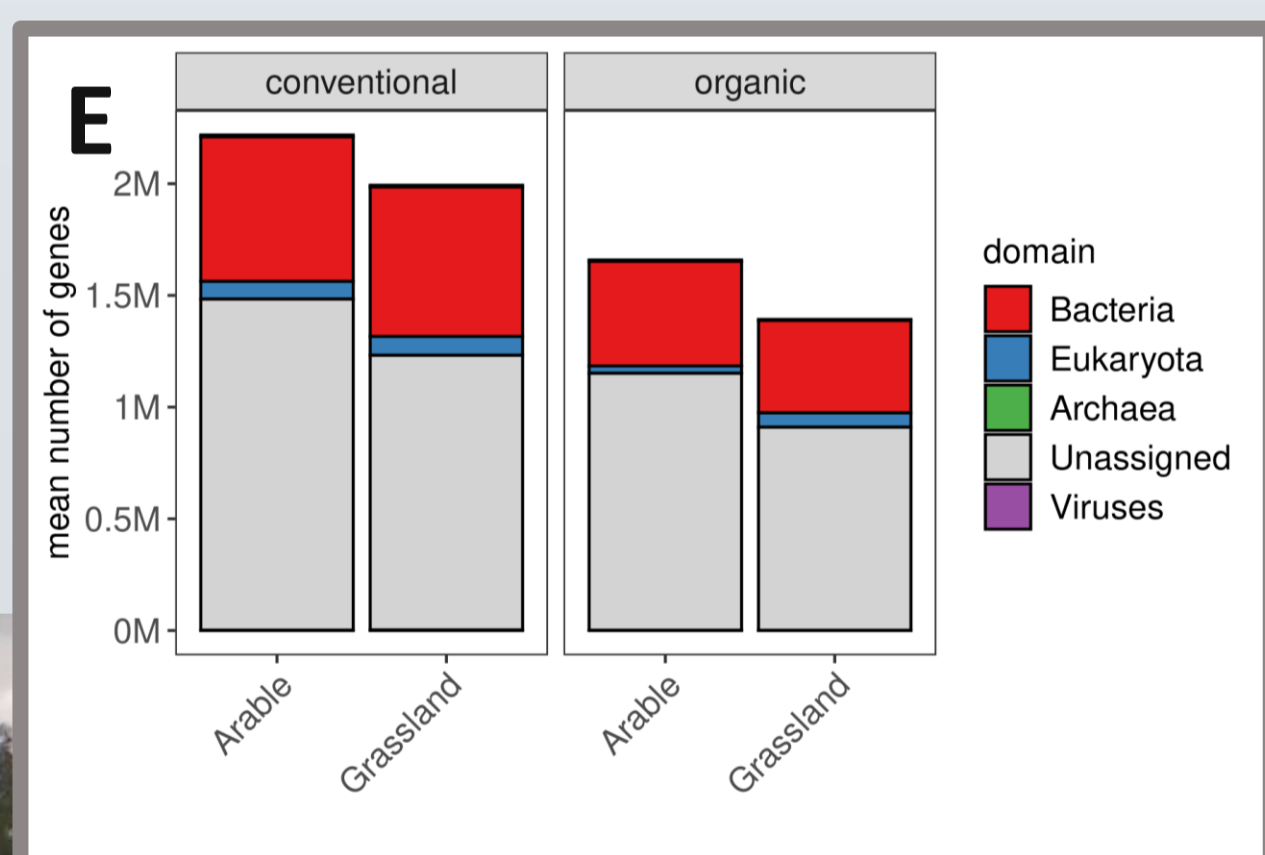
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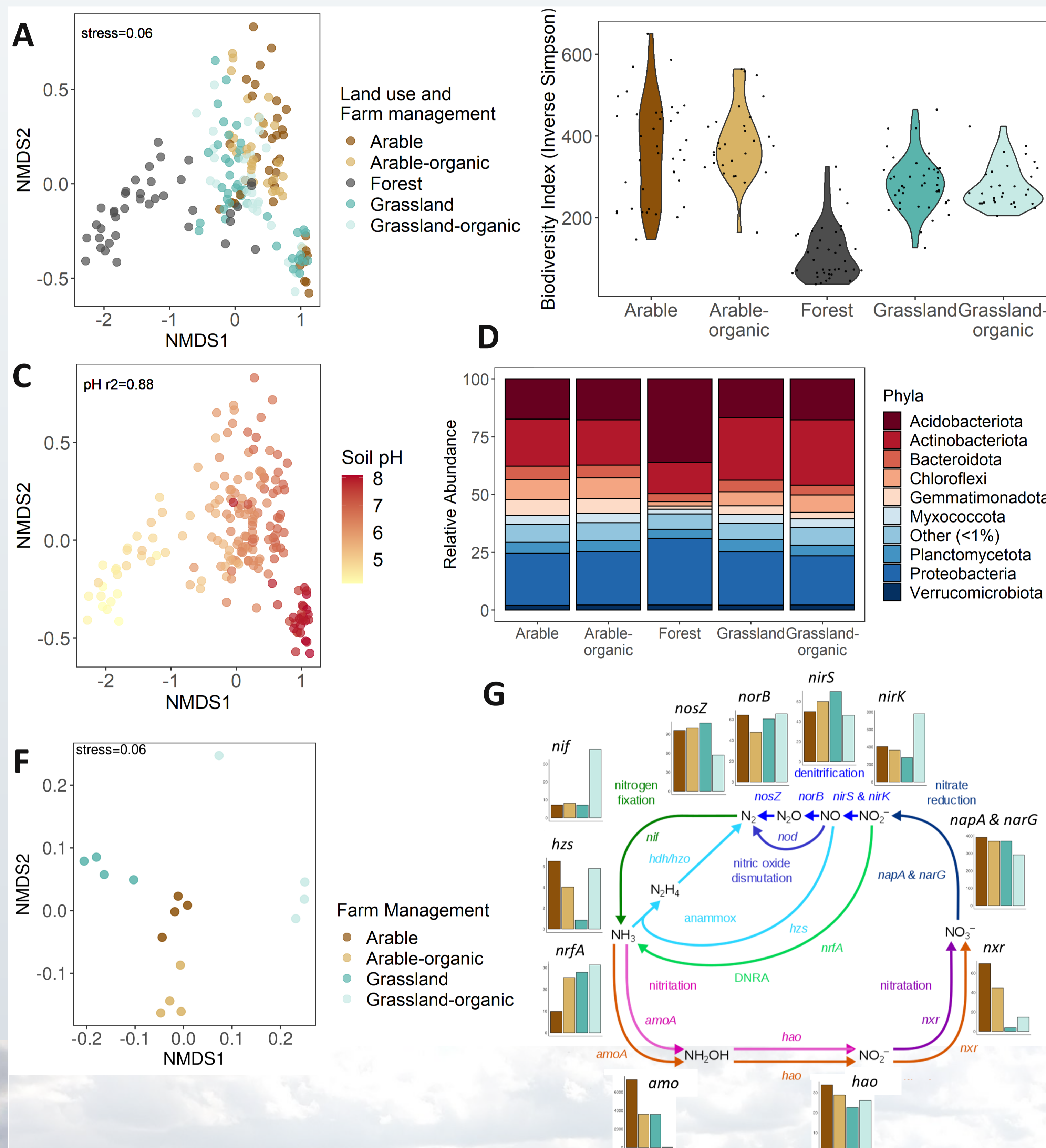
**Significance:** Soil microorganisms are critical for ecosystem functions. The preservation of soil biodiversity is part of the Green Deal. Here, we use high throughput sequencing to describe soil microbial biodiversity for Luxembourg arable, grassland, and forest soils. We investigated the influence of land use and conventional and organic farming on microbial community structure, biodiversity, and functional genetic potential. We show that bacterial biodiversity is highest in arable soil and lowest in forest soil, indicating that preserving biodiversity is more important than increasing soil biodiversity. Soil bacteria respond strongly to pH gradients associated with soil type, climate and farm management. Although much of soil's genetic potential remains unknown, our metatranscriptomic analysis indicates that the functional genetic potential of soil prokaryotes (bacteria and archaea) is sensitive to farm management.

## Research Questions

1. How do land use and farm management influence soil bacteria?
2. What are the main environmental controls on bacterial community and diversity? How important is farm management?
3. Does farm management (organic vs conventional) influence bacterial functional diversity and potential?



## Key Findings



**Land use:** influences community structure (A) and biodiversity (B) of even the major bacterial phyla (D). Highest biodiversity in arable soils (B).

**Soil pH:** the strongest control on structure and diversity (C).

**Farm management:** a small but significant influence on the microbial community (4%) but a strong effect via changes to soil pH (71%) (e.g., Mg), soil N, organic C, and K.

Most soil proteins cannot be functionally annotated, meaning that **the genetic potential of soil remains unknown (E)**.

Overall assigned gene expression (functional potential) differs more by individual fields than by land use or farm management (F).

Organic farm management **increases** or **decreases** the relative abundance of key gene families, such as **transporters**, **amino acid synthesis** and **N cycling**. Deeper analysis of the N cycle reveals nitrification potential is higher in arable, and organic grasslands have higher nitrite reduction and N-fixation potential (G).

## Methods

Soil samples were collected in 2018, 2019 and 2020 from 8 sites (2-3 land uses per site) in 4 soil types. Soil depth was 0-25 cm (arable), 0-15 cm (grassland), and 0-10 cm (forest) (n=4). Soils were frozen on site in liquid N<sub>2</sub> and stored at -80 °C until DNA and RNA extraction.

Prokaryote (bacteria and archaea) and fungal libraries were prepared from 16S rRNA and ITS (DNA), respectively. Libraries were sequenced using an Illumina MiSeq, processed using Usearch v11, grouped into OTUs, then taxonomic annotation was applied with Silva v 138.1. OTUs and biodiversity indices were associated with land use and environmental variables (soil type, pH, soil C:N) to assess controls. Fungal sequencing and analysis is in progress.

We estimated microbial functional potential by building soil metatranscriptomes for two land uses (arable and grassland) at an organic and conventional farm on the same soil type. Total soil RNA was extracted, enriched for prokaryotes, quality controlled, prepared into libraries, and pair-end sequenced on Illumina NextSeq (1.14Tb raw output). Reads were trimmed, co-assembled, mapped, then functional and taxonomic annotation were assigned using a variety of databases (KEGG, KO, FOAM, CAZY, NCyDB).

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## Policy Relevance:

1. Maintaining rather than increasing biodiversity is key for optimizing soil health.
2. Microbial community and functional biodiversity are sensitive to farm management in complex, interactive ways that require further molecular and functional research.

## Ongoing (project in progress) and future recommended research :

1. Fungal identity and biodiversity by land use and farm management, and fungal functional potential.
2. Field and experimental assays to link microbial biodiversity to function, in response to management.