



Conference Abstract

# From microbes to mammals: agriculture homogenizes pond biodiversity across different land-use types

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## Abstract

The diversity and composition of biological communities in ecosystems is typically linked to land use. Consequently, intensive agriculture has a strong influence on these patterns, including a reduction and homogenization of species diversity. Kettle holes (KH; also known as potholes) are small water bodies (<1 ha) typical to formerly glaciated land on the northern hemisphere, reaching densities up to several dozen per km<sup>2</sup>. They often function as drainage centers, linking various landscape elements in their surroundings and are hotspots of aquatic biodiversity in terrestrial landscapes. In the 1950s, the land use surrounding KH in Europe (e.g., Germany, Poland, The Netherlands) and North America (USA and Canada) shifted towards intensive agriculture, likely affecting the local and regional biodiversity of the KH.

Reports on the biodiversity of ponds in agricultural landscapes are scarce, and mostly focus on single taxa. We used deep eDNA amplicon sequencing of eukaryotic, bacterial,

and archaeal SSU rRNA genes to assess effects of land use on the overall species biodiversity of 67 KH in either arable fields, grasslands, or forests, all embedded in a landscape subjected to intensive agriculture for decades. These were later contrasted with Berlin city ponds. Metatranscriptomic analyses were conducted in parallel to investigate the taxonomic diversity and functionality of the active communities.

The amplicon data, >300 million reads, were analyzed with phyloFlash and Kraken2 using the SILVA\_SSU\_Ref database. Subsequently, the sequences were grouped based on taxonomy. The use of different annotation pipelines resulted in similar patterns. A subset of the data was analyzed using DADA2, generating amplicon sequence variants. Clustering the sequences as variants or according to taxonomy resulted in identical patterns, suggesting that broad taxonomical groupings provide sufficient resolution for general overviews.

Annotation of the eukaryotic community was challenging, as none of the currently available databases contains sufficient high-quality sequences to cover the entire phylogenetic breadth. To overcome this limitation, we used the SILVA\_SSU\_Parc database, which also contains short sequences excluded from SILVA\_SSU\_Ref, thus extending the taxonomic base of the analysis. The curated, high-quality PR2 eukaryotic database did not provide better results than the SILVA\_SSU\_Ref database. An incompatibility between the SILVA and classical taxonomic nomenclature resulted in a 'language barrier,' which could be partly resolved by using the parallel EMBL taxonomy provided in the SILVA databases. Nevertheless, we propose that short-read SSU-based eukaryotic taxonomic annotations should be validated by other means.

Despite such methodological limitations, our study demonstrates that deep amplicon sequencing of eDNA return a reliable picture of the biodiversity in complex ecosystems. Such information is sufficient to identify biodiversity patterns across the three domains of life and can serve to pinpoint taxonomic groups which should be investigated by a more detailed approach.

Our study concluded that long-term exposure to intensive agriculture results in biodiversity homogenization across diverse taxonomic groups, removing most differences in biodiversity patterns among land-use types. This outcome contrasts with biodiversity patterns associated with sediments of the KH, where temporal coverage by the eDNA analyses extends back to at least the onset of intensive agriculture. However, metatranscriptomic analyses, reflecting the distribution of activities rather than relative abundance, revealed temporal differences in the structure of the active community in KH of the investigated land-use types, matching times of field fertilization. Thus, even in a landscape where biodiversity has been homogenized, inputs from the surroundings result in short-term activity changes of different organisms. This needs to be considered when developing new management schemes needed to counteract the current biodiversity loss.

## **Keywords**

eDNA, eRNA, kettle holes, ponds, land use, bacteria, archaea, eukarya, metabarcoding, metatranscriptomics

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