



Soil Health Through the Lens of Macrofauna Diversity: Insights from Forests and Olive Groves

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Soil health is defined as the capacity of soil to function as a vital living system within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant, animal, and human health. The decline of soil health due to human impacts is an urgent ecological issue.

This study explores the diversity patterns and within-field variability of soil macrofauna communities across different land management systems in Portugal, employing a robust, multi-faceted approach. Two case studies were conducted to evaluate diversity patterns in forests (oak- and pine-dominated) and olive groves (intensive and extensive systems). Sampling was carried out in April and May 2024, utilizing a systematic grid design that incorporated two spatial methodologies: a k-means-based grid and random point allocation, resulting in 36–39 sampling points per system. To enhance comparability, an additional sampling point was included following the LUCAS methodology.

Macrofauna were identified morphologically at the order level, and abundance data were systematically recorded. For each land-use type, a subset of samples was analysed to quantify the biomass of individual taxonomic orders, providing deeper insights into the relevance of biomass as an ecological parameter. Species richness was assessed using incidence frequency data and compared across the various management systems, with a focus on Hill numbers.

Diversity estimates for the agricultural sites indicate that extensive agricultural systems support higher potential species diversity as sampling efforts increase, while intensive agricultural systems generally sustain a lower and less diverse macrofauna community. Similarly, forested site estimates reveal that oak-dominated habitats harbour significantly greater species diversity compared to pine-dominated habitats. The metabarcoding approach corroborated these patterns, providing complementary insights, and the correlation between high-throughput sequencing (HTS) reads and biomass is critically analysed.

This methodological framework underscores the profound impact of land-use practices on soil macrofauna diversity, highlighting their essential role in sustaining soil health and broader ecosystem functionality. By integrating soil macrofauna diversity into soil health assessments, this

study addresses a significant knowledge gap and offers practical guidance for developing improved soil management strategies that support sustainable land-use practices.