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Distribution of interaction networks and functions in a spatially heterogeneous system, case of agroforestry

Métaprogramme BIOSEFAIR

Project bilan : 2021 - 2024

March 2025

Agroforestry, broadly defined as the presence of trees in agricultural landscapes, is an example of mixed cropping systems that increase “planned biodiversity.” These trees, through their perennial structures, create a microclimate that varies over time, thereby modifying associated biodiversity and the activity of organisms through habitat diversification. However, few studies have examined the effects of agroforestry on soil organisms and the functions they perform.

The overall objective of this project is to highlight how ecological interaction networks and ecosystem functions respond to the spatial organization of the environment following the establishment of trees and grassy strips in arable fields. The main hypothesis is that modifying the spatial organization of plots by planting trees increases both biodiversity and multifunctionality in agricultural systems. The operational objectives are to: (A) characterize biodiversity changes within taxa and trophic groups, (B) infer interaction networks between studied organisms, and (C) establish relationships between ecological interaction networks, soil physicochemical functioning, and plant growth to better understand the multifunctionality of these plots.

This study was conducted at the Mediterranean Agroforestry Instrumented Platform under water stress (DIAMS), located south of Montpellier at an INRAE experimental station (UE Diascope, Mauguio). The site is a 5-ha factorial experimental design with three blocks. In each block, three treatments are compared: forest plantation plots, an agroforestry system, and arable cropland. Within the agroforestry system, we distinguish several habitats: (i) the tree row and its associated grassy strip, and (ii) the cultivated alley. Crops are managed under conventional low-input farming. The instrumented site allows continuous monitoring of incoming radiation at soil surface, soil temperature and moisture at different depths, root growth, and soil solution nutrient contents.

Crop growth and yields, as well as black locust growth and resource allocation strategies, were monitored. Morphological or molecular identification was used to characterize communities of soil engineers, surface-active macroarthropods, free-living and parasitic nematodes (plant-feeding and entomopathogenic), fungi and bacteria (both free-living and associated with parasitic nematodes), weeds, aerial phytophagous invertebrates, predators and parasitoids.

Interaction networks were reconstructed using field observations, either directly as bipartite interactions or inferred by combining taxon co-occurrence data with a knowledge graph of soil trophic interactions (from databases). The strength of responses across taxonomic and trophic groups was analyzed by comparing values obtained in agroforestry plots with those of agricultural and forest references. The response of the structure/composition of reconstructed modules was tested against the spatial organization of the agroforestry plots.

Experimental Design

The experiment is located at the Diascope experimental unit in Mauguio (Hérault, France). It consists of a three-block factorial design, each block including three treatments: agroforestry plots, annual monoculture plots (agricultural reference), and forest plantation plots (forest reference). In the agroforestry treatment, tree rows are spaced 17 m apart, with annual monocrops grown in between. Trees were planted on grassy strips sown in 2019, whose plant community composition has since developed naturally. The grassy strips are 2 m wide, with trees planted at 2 m intervals within the strip. In the forest treatment, trees are planted more densely, with 3 m between rows and 2 m between trees within a row. Plot sizes in this treatment range from 750 to 1,100 m² depending on the block. Agricultural plots range from 1,200 to 1,600 m². Cultivated plots are managed with low input levels and shallow tillage. The entire experimental setup covers about 5 ha and was established in 2017.

The woody species selected was black locust (*Robinia pseudoacacia*), chosen for its characteristics: deciduous foliage limiting light competition in winter, deep rooting system accessing water and nutrients from deeper horizons, nitrogen-fixing ability, and agronomic/economic interest through potential exploitation at different stages (e.g., timber). Crop rotation alternates cereals and legumes depending on climatic conditions, without being predefined. In 2023 (the main data collection year), soil tillage began in mid-January and spring barley was sown at the end of January.

Sampling Strategy

The experimental design is structured around three types of land use: annual monoculture, forest plantation, and agroforestry (rows and alleys). Within agroforestry plots, the grassy strip and cultivated alley are considered two distinct habitats where contrasting functions could occur. Four land-use treatments were thus studied: agricultural reference (C), forest reference (F), and within agroforestry, the under-tree strip (AF-LSA) and the cultivated alley (AF-C).

Five georeferenced points were chosen within each treatment and block, representing five field replicates. These points capture the variability of agricultural and forest plots. In agroforestry plots, sampling points in the grassy strip (LSA) and in the cultivated alley were aligned along the same transect to ensure correspondence between replicates. In total, for each variable measured (biodiversity, function), sixty values were obtained across the design.

<u>Results</u>

The results cover a wide range of organisms (soil, surface, and vegetation—herbaceous and woody) and the functions they perform.

Biodiversity

Overall results: Several hundred taxa were identified through various approaches: eDNA metabarcoding (bacteria, fungi, protists), Baermann funnel (nematodes), Macfadyen extraction (microarthropods), soil hand-sorting (macroinvertebrates), pitfall trapping (surface macroarthropods), colored pan traps (flying arthropods), leaf branch dissection (foliar arthropods), camera traps (mammals), and photography (pollinators). Together, these provided a nearly exhaustive inventory of taxa and trophic groups present during the vegetation growth season (some protocols were repeated three times in spring).

Mammals and herbivory: Rabbit activity-density was higher in agroforestry than in monoculture. Tree rows and grassy strips provide shelter and diverse resources. Rabbits were abundant, consumed barley seedlings, and reduced early vegetation cover, but barley stands recovered by stem elongation stage.

Flying insects: Invertebrate diversity was higher in tree-containing habitats (F and AF-LSA) than in cultivated zones (C and AF-C), confirming that increased planned plant diversity promotes spontaneous animal diversity. However, more individuals were collected in monoculture than in forest plots, with agroforestry showing intermediate values depending on the season. Movement between habitats is influenced by matrix permeability, likely higher in open cultivated areas.

Surface macrofauna: Tree presence did not significantly affect abundance or diversity of surface invertebrates, contrary to previous studies. Differences in abundance were sometimes observed between monoculture and forest, but not in diversity.

Free-living nematodes: Monoculture supported higher diversity of herbivores and lower diversity of predators compared to other treatments. For example, five herbivore taxa (Pratylenchus, Helicotylenchus, Trichodorus, Tylenchidae, Tylenchorhynchus) were found only in monoculture. Predator taxa (Discolaimus, Mononchus, Mylonchulus) were absent from monoculture. Potential predator–prey relationships reported in the literature may also occur at DIAMS.

Soil Functions

Weed regulation: Post-dispersal weed seed predation was highest in monoculture (~70%) and lowest in forest (~30%), with intermediate values in agroforestry. Carabid beetles were the main granivores.

Regulation of plant-parasitic nematodes: Laboratory assays showed higher suppressiveness in soils from vegetated habitats compared to monoculture soils, associated with nematode communities containing more predators.

Insect regulation: No native entomopathogenic nematodes (EPNs) were found, but entomopathogenic fungi (*Beauveria*) were more frequent in agroforestry soils. Experimental introductions of EPN strains suggested a positive effect of agroforestry on their parasitic cycle.

Organic matter dynamics: Litterbag tests with black locust leaves showed low mass loss (~6.8% after 5 weeks), likely due to drought. Significant differences in decomposition were observed between forest and agroforestry alleys, possibly linked to litter quality and herbivory.

Structural stability: Soil macroaggregates in monoculture had lower mean weight diameter (MWD) after wet sieving than those under trees. Infiltration rates were higher in grassy strips than in agroforestry alleys, highlighting a positive role of tree rows. Both stability and infiltration correlated strongly with soil C content.

CONCLUSIONS

These results still require integration to interconnect biodiversity and function patterns in agroecosystems. Some findings are published or in press, while others will contribute to broader reflections on biodiversity assessment during agroecosystem diversification under Mediterranean climatic constraints.

Valorisation

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Many organisms were collected during the IFOSSA sampling campaign, some outside the core expertise of project partners. Numerous specialists were consulted for identification. In addition, many people contributed to animal identification/validation via the collaborative platform iNaturalist. The project page gathering posted images is available here:

https://www.inaturalist.org/projects/diams_global?tab=species