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Impact of living mulches on belowground biodiversity and soil nutrient status of an organic apple orchard

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Abstract

The impact of living mulches established with three officinal plants (Alchemilla vulgaris, Fragaria vesca and Mentha x piperita) was assessed on the soil bacterial activity, the nematodes population, and the nutrient status of an organic apple orchard. The activity of bacterial microbiome associated with F. vesca was higher and utilising more C sources in comparison to other treatments. The living mulches did not affect the overall number of nematodes, but they modified in some cases the structure of the population: F. vesca induced the highest share of bacteria feeders and lowest number of herbivores and fungal feeders. The living mulches modified the availability of some nutrients and the pH.

Keywords: *Alchemilla vulgaris*, *Fragaria vesca*, *Mentha x piperita*, soil bacteria, nematodes

Introduction

Living mulches are a specific declination of intercrops for fruit crops, which were initially developed as a tool for weed control on the tree row, particularly for organically managed orchards (Granatstein, Sánchez 2009). However, recently they have been also evaluated for their possible exploitation as providers of other ecosystem services related to plant protection (e.g. biological control of soil-borne pathogens) (Greff et al. 2023) and the positive effect on overall aboveground insect community (Las Casas et al. 2022), as well as providers of additional income to the farmer (Mia et al. 2021). This study has thus assessed the impact of living mulches established with three medicinal plants on the soil environment in an organic apple orchard.

Material and Methods

The trial was conducted on a ten-year-old apple orchard (cv. Gala on M9 root-stock) established on a loamy sand soil (sand 78%+ silt 14%+ clay 4%) with 3.22% soil organic matter and pH 6.2. A Randomized Complete Block Design (RCBD) was laid out with four treatments of understorey soil management, each with three replications, including three living mulching species: *Alchemilla vulgaris*, *Fragaria vesca*, and *Mentha x piperita*. Natural soil cover was considered as control, and it was managed with three-time mowing during the growing season. Each replication consisted of 20 trees for a total row length of 30 m.

Soil samples for chemical and biological analyses were collected three times during the season with well-established living mulches (May, July and September in 2022). Sampling was performed using Egner's auger (2.5 cm diameter) collecting at least 10 subsamples at 0-20 cm depth within an area of 30 cm radius/distance around the apple tree trunk.

The soil samples were analysed in terms of pH, salinity, and the levels of the basic nutrients: N-NO3, N-NH4, P, and K. Soil microbial biodiversity and activity were determined using the BIOLOG® system and EcoPlates. Nematodes were extracted from soil using a modified Baermann method. All identified nematodes genera were assigned to five trophic groups (bacterial feeders, fungal feeders, omnivores, predators or plant feeders). The data were analysed by ANOVA and means differences tested with Tukey's test at $p \leq 0.05$. In case of not normal distribution, the non-parametric Kruskal-Wallis analysis with Fisher's least significant difference post hoc test was utilized, introducing the Benjamini-Hochberg correction.

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Results and Discussion

The living mulch species affected the concentration of some nutrients and chemical parameters, but not salinity, magnesium and N-NH4 levels. *F. vesca* induced an increase of the soil pH and P levels (also significantly increased in apple leaves, data not shown) in comparison to the control, and lowered N-NO3 levels. *A. vulgaris* induced a slight increase of N-NO3 levels and significantly decreased K levels when compared to control. *M. x piperita* resulted to increase K levels and decrease Ca concentration in soil. The observed changes in the soil nutrients were only marginally affecting apple leaves nutrient content: N, K and Mg content in leaves were not affected, while negligible changes occurred for Ca (data not shown). The modifications of the soil chemical characteristics could be the effect of both plants growth and microbial activity (see below), but also the result of season-dependent changes in plant uptake and microbial activity. The observed decrease of soil pH with the living mulches could be ascribed to the higher bacterial activity (i.e. respiration), but also to an increased release of root exudates by the living mulch species. The balance between microbial activity and plant uptake has been found shaping the availability of ions (e.g. Ca2+, K+, NO3-), which is also related to electrical conductivity (salinity) and pH as well (Raddatz et al. 2020). The modification of the soil pH could have also affected the availability of P: soil covered by *F. vesca*, characterized by high bacterial activity and presence of genera associated to mycorrhizal fungi (data not shown), known for their P solubilization capacity, showed also the highest content of phosphorus.

The highest average bacterial activity was observed in autumn (Table 1).

Table 1: Effect of living mulching and season on soil bacterial activity (AWCD) and biodiversity indices (Shannon-Weaver coefficient – H', and substrate richness - S). Different letters show statistically significant differences for p≤0.05.

Treatment	AWCD	H'	S
Control	1.54 b	3.27 a	28.67 a
A. vulgaris	1.45 b	3.15 a	24.44 b
F. vesca	1.64 a	3.24 a	27.78 a
M. piperita	1.55 ab	3.20 a	26.67 a
p-value	0.00504	0.064	0.000355

F. vesca induced a significantly higher activity compared to control or *A. vulgaris* treatment, and soil from *M. x piperita* having an intermediate activity. Substrate richness was significantly lower in *A. vulgaris* treatment in comparison to the other treatments. No statistically significant differences between treatments were observed for H Index calculated on microbial metabolic potential, but a higher diversity (S) was observed in control compared to other treatments, particularly *A. vulgaris*. Such modifications could be related to the process of soil microbiome selection by the living mulch species mediated probably by the specific secondary metabolites or essential oils released by the root systems into the soil environment (Misra et al. 2019; Santoro et al. 2016).

Between 370 and 524 nematodes individuals per sample were isolated from the soil samples collected during summer sampling timepoint. The total number of nematodes was not affected by the treatment, although some differences were observed in the relative abundance of bacterivores and fungivores feeding groups (Table 2). *F. vesca* induced and increase of bacterivores compared to control and *A. vulgaris*, while the latter showed significantly higher share of fungi feeders than *F. vesca*.

Table 2: Total nematodes population and trophic group abundance of tree understorey soil managed with living mulch species or natural cover of an organic apple orchard. Means±SEM. Different letters show statistically significant differences between treatments for p≤ 0.05.

Treatment	Bacterivores	Fungivores	Herbivores	Omnivores	Predators
A. vulgaris	48.8±1.0 b	26.3±2.5 a	19.3±2.8 a	3.0±1.8 a	0.9±1.2 a
F. vesca	75.5±2.3 a	7.8±2.4 b	9.2±1.9 a	0.7±1.1 a	4.2±1.9 a
M. piperita	65.4±3.2 ab	14.8±2.8 ab	15.7±3.3 a	2.5±1.6 a	1.6±1.7 a
Control	48.5±3.6 b	12.2±2.6 ab	24.1±2.5 a	4.5±2.1 a	9.1±2.7 a

The bacteria feeders were the most abundant trophic group, which is common for various terrestrial environments (Wilschut, Geisen 2021). However, the highest share of bacteria feeders and lowest number of herbivores and fungal feeders with *F. vesca* mulching can be considered a positive effect of this species, unlikely to especially mulching with *A. vulgaris*.

In conclusion, it is confirmed that the introduction of multifunctional living mulches based on officinal plants is inducing changes to the soil genetic and functional biodiversity, which result in modification of the soil chemical properties. These modifications could thus deliver ecosystem services related to nutrients cycling and plant protection, which can directly impact the major crop.

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