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Effect of Olive Grove Management by Groundcovers on Soil Microbiological Biomass

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Abstract: Soil management strategies in agricultural environments allowing for successful world food supply through practices that are respectful with the ecological balance of the agroecosystems are needed. Thus, the interest in the use of groundcovers in integrated production has been increasing due to the possibility to meet profitability and sustainability. This work aimed to compare four soil management conditions in an olive grove: annual spontaneous vegetation, grass and legume groundcovers, and conventional tillage, in terms of biomass generated by microorganisms in soil and plant roots. Population sizes of fungi, bacteria and nematodes, and soil aggregate stability were estimated in each condition. Regarding the contrast between plant roots and soil, increases in fungi, bacteria and nematodes correlated with plant roots and, consequently, with increases in microbiological biomass in the olive grove. Moreover, higher stability of soil aggregates was observed in the three groundcovers, which may be associated with increases obtained in fungi and bacteria in these conditions. Among groundcovers, only that of legume pointed out a slight increase in fungi and bacteria in soil and plant roots, which may indicate the need for long-term studies of this kind to allow for adequate establishment of microbiological populations.

Key words: land-use change, soil biology, sustainable agriculture, agroecology, soil health, microorganism, food supply, cover crop

1. Introduction

Agriculture copes with relevant challenges, the most important of them being the production of enough nutriment to meet global demand of a rapidly growing world population [1-3] trying to overcome difficulties imposed by climate change as rising temperatures and water scarcity. To meet this challenge, agricultural production must increase at the same time that the agroecosystems involved are preserved and their ecological biodiversity maintained [1, 2, 4]. Consequently, farmers should resort to new alternative strategies to enhance productivity from natural resources through sustainable land use [2, 5, 6] that may have beneficial long-term effects on quality and quantity of agricultural products intended for worldwide supply.

One of these biological strategies compatible with environmental conservation and protection is the use of groundcovers [7, 8]. This method constitutes a re-emerging trend in cultivation techniques that can be implemented in woody crops and others to improve traditional agricultural practices, often replacing long-established fallows, and taking into account the sustainability of the agricultural systems [5, 7, 9]. The interest in the inclusion of groundcovers in integrated production of agricultural systems has been progressively increasing due to population concern related to the use of herbicides, inorganic fertilizers, and other agrochemicals, and their interactions with soil nutrient availability and the possibility of plant phytotoxicity [10]. Groundcovers have numerous advantages [8, 9, 11], which can contribute to

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successfully satisfy both farm profitability and social demands.

The convenience to use groundcovers would be mainly supported by their potential beneficial effects on soil, cultivated plants and the environment [5, 8, 9, 12, 13]. These include (i) improvement of soil structure because of increments in soil organic matter and porosity due to growth and activity of plant roots, (ii) covering of the surface to avoid soil displacement by erosion, (iii) increment in soil fertility because of higher organic matter contents, prevention of nutrient losses, along with carbon accumulation, nitrogen fixation, and supply of other nutrients, (iv) enhancement of biomass and biodiversity of soil microbial populations due to improved nutrient contents and aeration of soil habitats, (v) weed suppression, favouring the growth of intended crops, (vi) biocontrol activity against plant diseases and pests, maintaining crop health, (vii) increment in the biodiversity of the agroecosystems, sheltering plant pollinators, natural predators as well as soil fauna, and (viii) reduction of greenhouse gases and/or mitigation of climate change by promoting carbon sequestration.

In spite of that, there remains insufficient data on the effect of management practices as groundcovers on soil microbiological biomass and/or the soil biology.

The main objective of this work was the comparison of four soil management strategies, three different types of groundcovers and conventional tillage, in terms of generated microbiological biomass of fungi, bacteria and nematodes. The plot was located in an experimental olive grove in Central Spain, with marl-gypsiferous soil under semi-arid climate. Some preliminary study of this kind was recently described [14], mainly taking into account roots and aerial parts of the plants. This work focused on production of microbiological biomass in soil and plant roots, comparing the results in relation to soil aggregates.

2. Material and Methods

The study was performed in an experimental olive grove (*Olea europaea* L.) located in Central Spain, in southern Madrid, covering an area of about 3 ha. The cultivar was Cornicabra, the most widely grown cultivar in Central Spain, drought tolerant and cold resistant [15]. The soil was classified as Haplic Gypsisol [16], with a xeric moisture regime. A high silt percentage was measured, with a moderate to low cation exchange capacity, moderate to high electrical conductivity and low soil organic carbon and total nitrogen contents.

The olive grove was rainfed and no emergency irrigation was applied during the experimental period. The mean annual precipitation was approximately 284 mm with high inter and intra-annual variability, and the reference evapotranspiration (ET0 Penman-Monteith) was 1200 mm. The climate was Mediterranean semiarid, with long hot summers and cold winters, and a mean annual temperature of 13.6°C.

The experimental plots making up the olive grove were located so that there was a total number of 16 grouped in 4 blocks, each of them containing 4 soil management treatments: three types of groundcovers, and conventional tillage. The groundcovers were the following: annual spontaneous plant cover, permanent grass cover (*Brachypodium distachyon*), and annual legume cover (*Vicia ervilia*). Control plots (without groundcovers) were managed with conventional tillage. The study was carried out for 4 years (2014-2018). In this work, the results from the last field season (2018) were presented.

From all the plots, composite soil samples and plant root samples were taken either at 0-10 cm depth for a later microbiological analysis, or at 0-5 and 5-10 cm depth for soil aggregate stability tests. Root sampling consisted of the random collection of at least 10 plants from each plot, maintained without contact between roots and aerial parts. Microbiological analyses from each plant sample took place after cutting the roots from the aerial parts under aseptic conditions. Samples of either soil and/or plant roots from each block (the 4 treatments) were analysed at the same time, for comparative purposes.

Briefly, population sizes of fungi and bacteria from each soil or root sample were estimated from 1:10 (w/v) in phosphate buffered saline (PBS) solution, shaken at 200 rpm during 30 min, and plating of serial ten-fold dilutions onto the general media Potato Dextrose Agar (PDA) with streptomycin 0.5% and Nutrient Agar (NA) for fungi and bacteria, respectively. Population sizes of fungi and bacteria were estimated by plate counts of isolated colonies after incubation at 25°C during 48 h for bacteria and 72 h for fungi [17] (Fig. 1A and 1B).

Population sizes of nematodes from each soil sample were estimated from 100 g mixed with sterile water during 48 h to extract the nematodes by the Baermann funnel technique and direct counting of individuals with a stereoscope [18] (Fig. 1C and 1D). From each root sample, the remaining PBS suspensions with root material passed through a sieve, which allowed for the direct counting of nematodes with a stereoscope.

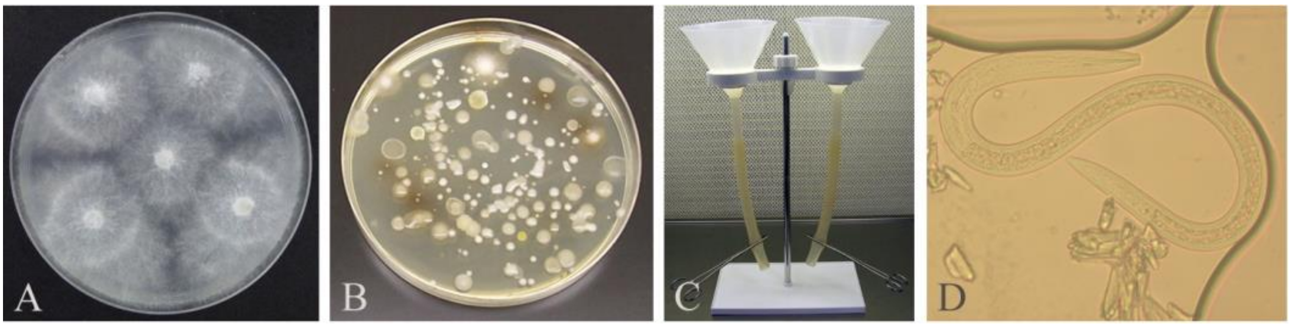

Fig. 1 Microbiological analyses in the experimental plots of the olive grove. Fungi, bacteria and nematodes from soil and plant roots from groundcovers were either isolated and counted on the respective solid general media for A) fungi, B) bacteria, or C) extracted by the Baermann funnel technique and D) counted (total amplification×400).

Soil aggregates from each plot were analysed by the counting number drop test [19].

3. Results and Discussion

In soil, fungi and bacteria presented slightly higher population sizes in legume groundcover than in the rest of soil management strategies (Table 1). However, nematode values were in some measure higher in conventional tillage than in the rest of treatments, with a great variability between blocks (Table 1). No significant differences were found regarding the four soil treatments for the three types of microorganisms.

In plant roots, the population sizes of epiphytic fungi and bacteria were moderately higher in legume groundcover than in the other soil management conditions (Table 1), highlighting the different groundcovers with respect to conventional tillage in the case of fungi (Table 1). In the same way, the numbers of epiphytic nematodes were higher with groundcovers compared to conventional tillage, especially in spontaneous vegetation and grass covers, although with a high variability between blocks (Table 1). No significant differences were found among the four soil management strategies for the three groups of microorganisms.

However, when comparing the population sizes obtained in soil and plant roots, higher values in plant roots were observed than in soil for fungi, bacteria and nematodes (Table 1). In the case of nematodes, these differences were up to approximately two orders of magnitude (Table 1). With respect to soil aggregate stability tests, values were also higher in groundcovers than in conventional tillage (Table 1), although without statistically significant differences.

The observation that population sizes of fungi, bacteria and nematodes were consistently greater when obtained from plant root analyses with respect to soil in each of the four soil treatments in the olive grove was in accordance to previous studies [13, 14, 20], pointing out an invariable increment in microbiological biomass.

Table 1 Comparison of the four soil management strategies considered in the experimental plots of the olive grove.

Data from 2018 sampling		Spontaneous cover	Grass cover	Legume cover	Conventional tillage
Soil	Fungi log (CFU[1]/g)	4.05±0.10	4.29±0.24	4.76±0.12	4.37±0.36
	Bacteria log (CFU/g)	6.31±0.20	6.57±0.17	7.08±0.07	6.68±0.24
	Nematodes (indiv.[2]/g)	0.55±0.36	0.41±0.21	0.62±0.27	0.75±0.69
	Aggr.[3](CND)[4] 0-5 cm depth	25.6±12.4	19.2±8.8	16.8±6.5	10.2±1.6
	Aggr. (CND) 5-10 cm depth	17.4±7.4	15.3±9.1	16.1±7.1	10.6±2.7
Plant roots	Fungi log (CFU/g)	5.08±0.71	5.14±0.37	5.38±0.54	4.43±0.29
	Bacteria log (CFU/g)	6.98±0.48	7.30±0.24	7.74±0.19	7.18±0.63
	Nematodes (indiv./g)	85.87±98.41	83.44±82.39	30.73±52.45	17.84±25.13

[1] CFU: colony-forming units; [2] indiv.: individuals; [3] Aggr.: Aggregates; [4] CND: counting number drop. All microbiological analyses were at 0-10 cm depth. Values are the means for four replicates±standard deviation (n = 4). No significant differences were found among the four treatments for the three groups of microorganisms.

One of the main reasons for that would be the release of nutrients by plant root exudates such as sugars, aminoacids, flavonoids, proteins, and fatty acids that directly or indirectly enhance growth of microorganisms, and the fact that roots improve aeration and water retention in soil [8, 21]. Groundcovers can contribute with organic substrates to the soil not only by root exudates but also from plant residues, which may have an additional effect on soil microbiological communities and their interactions [10].

On the other hand, data suggested that population sizes of microorganisms such as fungi and bacteria might have an influence in soil aggregate stability, so that values in the plots with groundcovers were slightly higher in relation to conventional tillage. Soil microorganisms favour soil aggregation processes due to the activity of their biomass and secretions [21]. Thus, bacterial exopolysaccharides produced in colonies and biofilms would be responsible for aggregating soil particles, and filamentous fungal morphologies would be effective too. In that sense, fungal hyphae may be stabilizing soil macroaggregates, while bacteria, polysaccharides and inorganic materials may be stabilizing soil microaggregates [22]. Plant roots would be actively involved in this process by supporting microbial communities, helping stabilize soil structure and controlling erosion [21].

From the comparison of the four soil management strategies, there was a slight trend in legume groundcover to host higher population sizes of fungi, bacteria and nematodes in both soil and plant roots. This might be due to that legume cover can supply more nitrogen to crops from biological fixation than other kind of cover or than tillage. In fact, under certain conditions, legume groundcovers favoured the increment of microbial biomass levels based on nitrogen contents compared to no groundcovers [21, 23], providing benefits to both soil fertility and the environment. However, in general, effects of groundcovers on the generated microbiological biomass are variable [14].

The apparently limited effects of groundcovers in this work may have been due, among other factors, to an insufficient duration of the experimental period and the particular conditions of the soil and climate, which did not favour microbial activity or plant production. Thus, other authors [24] described significant changes on soil indicators associated to soil quality in comparison to conventional tillage after 8 years of groundcover establishment, mainly reduced erosion, improved soil physical and chemical properties, modification of structure and diversity of soil bacterial communities, and increment of the microbial functional activities. Moreover, beneficial changes in soil can persist during long-term management, as 10 and 22 years of planting a monoculture groundcover increased bacterial gene abundance and diversity down to a depth of 60 cm in soil compared to no groundcover control treatment [10, 25]. Therefore, although groundcovers can have variable effects on soil microbial properties depending on local limitations, they can promote biological soil health by enhancing microbial community abundance compared to no groundcover conditions [25]. As soil characteristics influence soil microbial communities, any potential manipulation of soil factors through management will consequently have an effect on soil microbiota, and therefore, one of the most promising solutions to restore degraded soils and meet the rapidly increasing needs of an expanding global population would be to enhance the understanding of soil biology [21, 23, 24]. Overall, soil management by groundcovers constitute a suitable strategy for sustainable intensification of agriculture, soil conservation and the many aspects of soil biology where groundcovers can contribute with global beneficial effects in the agroecosystems.

4. Conclusions

The presence of groundcover roots can be related to increases in population sizes of fungi, bacteria and nematodes and, therefore, to increases in microbiological biomass in the olive grove.

Relatively higher stability of soil aggregates in the plots with groundcovers might correlate with increases in population sizes of fungi and bacteria in these experimental growing conditions. In these cases, fungal hyphae might be stabilizing soil macroaggregates, while bacteria, polysaccharides and inorganic materials might be stabilizing soil microaggregates.

Among groundcovers in the olive grove, only that of legume seems to have favoured a slight increase in the population sizes of fungi and bacteria, both in soil and plant roots.

Soil management by groundcovers maintained over time would allow for an adequate establishment of microbiological populations to take place, revealing the need for long-term studies of this kind in agricultural environments.

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References

[1] FAO, The future of food and agriculture — Trends and challenges, Rome, 2017, available online at: http://www.fao.org/3/a-i6583e.pdf.

[2] FAO, The future of food and agriculture — Alternative pathways to 2050, Summary version, Rome, 2018, p. 60, available online at: http://www.fao.org/3/CA1553EN/ca1553en.pdf.

[3] O. Calicioglu, A. Flammini, S. Bracco, L. Bellù and R. Sims, The future challenges of food and agriculture: An integrated analysis of trends and solutions, *Sustainability* 11 (2019) 222, doi: 10.3390/su11010222.

[4] FAO, The state of the world's land and water resources for food and agriculture (SOLAW) — Managing systems at risk, Food and Agriculture Organization of United Nations, Rome and Earthscan, London, 2011, available online at: http://www.fao.org/3/a-i1688e.pdf.

[5] A. Wezel, M. Casagrande, F. Celette, J. F. Vian, A. Ferrer and J. Peigné, Agroecological practices for sustainable agriculture: A review, *Agronomy for Sustainable Development* 34 (2014) 1-20.

[6] L. Carlisle, M. Montenegro de Wit, M. S. DeLonge, A. Iles, A. Calo, C. Getz, J. Ory, K. Munden-Dixon, R. Galt, B. Melone, R. Knox and D. Press, Transitioning to sustainable agriculture requires growing and sustaining an ecologically skilled workforce, *Front. Sustain. Food Syst.* 3 (2019) 96, doi: 10.3389/fsufs.2019.00096.

[7] A. Clark (Ed.), *Managing Cover Crops Profitably* (3rd ed.), Handbook Series Book 9, Beltsville, MD, The Sustainable Agriculture Network, 2007.

[8] P. Sharma, A. Singh, C. S. Kahlon, A. S. Brar, K. K. Grover, M. Dia and R. L. Steiner, The role of cover crops towards sustainable soil health and agriculture — A review paper, *American Journal of Plant Sciences*, 9, (2018) 1935-1951, doi: https://doi.org/10.4236/ajps.2018.99140.

[9] R. A. Wittwer, B. Dorn, W. Jossi, M. G. A. van der Heijden, Cover crops support ecological intensification of

arable cropping systems, *Scientific Reports* 7 (2017) 41911, doi: 10.1038/srep41911.

[10] A. Castellano-Hinojosa and S. L. Strauss, Impact of cover crops on soil microbiome of tree crops, *Microorganisms* 8 (2020) 328, doi: 10.3390/microorganisms8030328.

[11] B. Sastre, C. Barbero-Sierra, R. Bienes, M. J. Marqués and A. García-Díaz, Soil loss in an olive grove in Central Spain under cover crops and tillage treatments, and farmer perceptions, *Journal of Soils and Sediments* 17 (3) (2017) 873-888, doi: https://doi.org/10.1007/s11368-016-1589-9.

[12] B. Sastre, M. J. Marqués, A. García-Díaz and R. Bienes, Three years of management with cover crops protecting sloping olive groves soils, carbon and water effects on gypsiferous soil, *Catena* 171 (2018) 115-124.

[13] D. M. Finney, J. S. Buyer and J. P. Kaye, Living cover crops have immediate impacts on soil microbial community structure and function, *J. Soil and Water Conservation* 72 (4) (2017) 361-373, doi: 10.2489/jswc.72.4.361.

[14] B. Sastre, B. Álvarez, O. Antón, M. A. Pérez, M. J. Marqués, R. Bienes and A. García-Díaz, Groundcovers in olive groves in semiarid climates: are they always beneficial? *Water* 12 (2020) 2230, doi: 10.3390/w12082230.

[15] L. Rallo, D. Barranco, J. M. Caballero, C. del Río, A. Martín, J. Tous and I. Trujillo, Variedades de olivo en España [Olive tree cultivars in Spain], Junta de Andalucía, MAPA y Ediciones Mundi-Prensa, Madrid, 2005.

[16] IUSS Working Group WRB, World Reference Base for Soil Resources, International soil classification system for naming soils and creating legends for soil maps, World Soil Resources Reports No. 106, FAO, Rome, 2014.

[17] N. Varghese and P. P. Joy, *Microbiology Laboratory Manual*, Pineapple Research Station, Kerala Agricultural University, 2014.

[18] EPPO, PM 7/119 (1) Nematode extraction, *EPPO Bulletin* 43 (2013) (3) 471-495.

[19] A. C. Imeson and M. Vis, Assessing soil aggregate stability by water-drop impact and ultrasonic dispersion, *Geoderma* 34 (1984) 185-200.

[20] M. D. McDaniel, L. K. Tiemann and A. S. Grandy, Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis, *Ecological Applications* 24 (2014) (3) 560-570.

[21] R. M. Lehman, C. A. Cambardella, D. E. Stott, V. Acosta-Martinez, D. K. Manter, J. S. Buyer, J. E. Maul, J. L. Smith, H. P. Collins, J. J. Halvorson, R. J. Kremer, J. G. Lundgren, T. F. Ducey, V. L. Jin and D. L. Karlen, Understanding and enhancing soil biological health: the solution for reversing soil degradation, *Sustainability* 7 (2015) 988-1027, doi: 10.3390/su7010988.

[22] J. M. Tisdall, Possible role of soil microorganisms in aggregation in soils, *Plant Soil* 159 (1994) 115.

[23] I. Muhammada, J. Wang, U. M. Sainju, S. Zhangb, F. Zhao and A. Khan, Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis, *Geoderma* 381 (2021) 114696.

[24] L. F. Arias-Giraldo, G. Guzmán, M. Montes-Borrego, D. Gramaje, J. A. Gómez and B. B. Landa, Going beyond soil conservation with the use of cover crops in Mediterranean sloping olive orchards, *Agronomy* 11 (2021) 1387, doi: https://doi.org/10.3390/agronomy11071387.

[25] C. Liu, Y. Jin, Y. Hu, J. Tang, Q. Xiong, M. Xu, F. Bibi and K. C. Beng, Drivers of soil bacterial community structure and diversity in tropical agroforestry systems, *Agric. Ecosyst. Environ.* 278 (2019) 24-34.