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First results on the evaluation of the ground-cover biodiversity in an agroforestry poultry system

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The first results on the impact of hen grazing on ground cover composition and biodiversity of two hazelnut orchards (sites A and B) in the southern Italy are presented. The selected sites were characterized by different size and environmental conditions that were, respectively for A and B: area, 4 and 5 ha; altitude, 525 and 660 m a.s.l.; annual mean temperature, +12°C and +13°C; annual precipitation: 730 and 780 mm. Besides, ground covers of the sites were different for plant species presence and distribution. In each site three 120 m^2 areas were considered, each of which was provided with a mobile coop (5 hens/m^2) to house during the night ten 25-week-old commercial egg-laying chickens. The trial started the first decade of May 2019. The use of hen grazing affected quantitatively and qualitatively the herbaceous stratum of the study sites, as the number of *taxa* composing the ground cover decreased in both sites, whereas the relative frequencies varied (increased or decreased) according to the *taxon*. By computing different indices of alpha diversity, we observed significant differences in species richness and diversity after hen grazing only in one site. However, the value of the qualitative Sørensen index showed a high overlap in the assemblage of ground cover before and after hen grazing in both sites. By comparing the sites, we found that, at the beginning of the trial, the ground cover of one site was richer and more diverse compared to the other site; however, the reverse situation occurred after hen grazing. Overall, our results indicate that integrating fruit trees with poultry may play a positive role in an agroforestry context, mainly in terms of control of vegetation growth.

Keywords: agroforestry; grazing; poultry; ground-cover; biodiversity

1 Introduction

It is well recognized that Europe's biodiversity is to a large part inextricably linked to agroforestry practices (Torralba et al., 2016): in fact, the mosaic of habitats featuring several European landscapes favoured a diversity of plant and animal species thanks to the millennial transformation process operated by humans on ecosystems by cutting, fire and grazing. These agro-silvo-pastoral activities play, today more than ever, a fundamental role in the conservation of biodiversity, as long as they are carried out in a sustainable way (Freschi et al., 2015 b). When properly designed, these practices can provide several advantages, such as reduced pest management inputs, increased crop production, diversification of farm income, reduced soil erosion, improved water quality and reduced water consumption (Angima, 2009; Cosentino et al., 2015). This is particularly true for grazing, whose proper management may positively affect botanical and faunal biodiversity (Rook and Tallowin, 2003; Tallowin et al., 2005; Freschi et al., 2015 a). In fact, it has been reported that moderate levels of grazing are important for promoting richness and diversity of plants and invertebrates, which, in turn, support native animals, including many that are listed as threatened and endangered (Freschi et al.,

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2015 a). Compared to conventional intensive systems, raising poultry on free-range offers a potential to enhance animal welfare, as it provides birds with sunlight, fresh air, ample space and nutrients, and allows them to express natural behaviours (Berg, 2002; Sossidou et al., 2011; Mohammed et al., 2013). Besides, it offers many advantages in terms of farm soil fertility, disease prevention, weed control, farm diversity, environmental sustainability and farm profitability (Sossidou et al., 2011; Liu et al., 2013). In an agroforestry context, where woody perennials (trees or shrubs) are integrated with crops and/or livestock on the same land unit to optimize beneficial interactions between the woody and other components (Nair, 1993; Burgess et al., 2015), the aforementioned advantages of raising poultry on free-range are particularly important (O'Brian et al., 2006; Stobbelaar and Hendriks, 2011; Smith et al., 2013). An interesting approach is to integrate hen grazing within high value fruit trees agroforestry systems, such as olive, hazelnut, walnut, almond, chestnut, apple and pear systems. This may offer an additional source of income while providing weed control and fertilization, thus lowering costs and impact of the management. However, combining the above productive aspects with environmental enhancement should be a priority objective for managing the grazing resources in an agroforestry system in the third millennium. To date, there are few studies that have investigated the association between hen grazing and orchards (Rosati et al., 2016; Timmermans and Bestman, 2016). Nevertheless, none of these studies assessed the impact of this association from a plant biodiversity perspective. Therefore, the present study was designed to investigate the impact of hen grazing integrated into hazelnut orchards on the ground cover composition and biodiversity.

2 Material and methods

2.1 Study sites

The present study was conducted in May 2019 in two different sites of cultivated hazelnuts (*Corylus avellana*, cultivar 'Tonda di Giffoni', 3 × 5 m tree spacing) located in the Basilicata region, South of Italy. The first site (Site A; 40°49'57.62''N - 15°42'44.42''E) has an area of approximately 4 ha and lies within 500 to 550 m a.s.l.. Its mean annual precipitation is 730 mm, whereas its temperature is +12.5°C. In the second site (Site B; 40°24'16.76''N - 15°43'44.80''E), which spans approximately 5 ha, the elevation is 660 m a.s.l., and the mean annual precipitation and the temperature are 780 mm and 13.1 °C, respectively.

2.2 Housing and feeding of hens

Three homogeneous areas sized up 40 x 3 m were selected in each site and delimited by electrified net. Each area was provided with mobile coop (5 hens/m^2) to house during the night 10 hens ageing 25 weeks and belonging to the same commercial egg-laying genotype. Hens were fed on the same basal diet, a commercial feed with the following characteristics on a DM basis: crude protein 17.00%, crude fat 4.50%, crude fiber 4.50%, crude ash 13.00%, Lys 0.90%, Met 0.36%, Ca 4.00%, P 0.56%, Na 0.17%, Vitamin A 6,000 IU, Vitamin D3 1,800 IU. Besides, the hens were given access to the pasture for 12 hours a day (from 07:00 AM to 07:00 PM). Feed and water were supplied externally to the mobile coop by manual bell feeders and automatic drinkers.

2.3 Plant species composition of ground cover

The botanical composition of the herbaceous stratum of each site was evaluated before and after hen grazing by using the quadrat method (Bonham, 1989, modified by Rizzardini et al., 2019). Hen grazing lasted three weeks. Briefly, in each site two 20 m long transects were used to sample vegetation. Along each transect, separated from one another by 1 m, plant species were listed in 20 quadrats (sized 1 m^2) arranged at a distance of 1 m from each other. The collected specimens were identified according to Flora Europaea (Tutin et al., 1993).

2.4 Statistical analysis

In each site, data on identified plant species composing the ground cover before and after hen grazing were used to calculate the relative percentage of a *taxon* (Freschi et al., 2016, 2017). The same data were used to compute the following alpha diversity indices: 1) species richness (*D*), for which the higher the value the greater the richness (Margalef, 1958); 2) species diversity (*H*) (Shannon and Weaver, 1949), whose value usually ranges from 1.5 to 3.5 and often does not exceed 4 (Margalef, 1972); 3) species evenness (*E*) (Buzas and Gibson, 1969), whose value ranges from 0 to 1, where 1 indicates that all the food items are used to an equal extent. Differences in species richness, diversity, and evenness were tested at intra- (comparison of the two different sampling periods in each site) and inter-site (comparison of the two same sampling periods between sites) level by using the Student ttest. To measure the extent of differentiation in species composition in each site and between sites, we used the Sørensen index (CS) (Sørensen, 1948), which considers presence/absence data and whose value ranges from 0 (complete dissimilarity) to 1 (complete similarity).

3 Results and discussion

3.1 Plant species composition of ground cover in the sites before and after hen grazing

Table 1 shows the relative percentage of plant species identified in Site A and B before and after hen grazing. Overall eighty-five plant *taxa* were identified in the sites during the present study. Forty-six plant species composed the herbaceous stratum of site A at the beginning of the trial. Almost half of them (46%) were observed in low percentages (<1%); among them, there were *Daucus carota*, *Picris hieracioides*, *Malva silvestris*, *Trifolium campestre*. Other *taxa* were instead observed at higher percentages: for instance, the following ten *taxa* accounted for 57.6% of the all observed species composing the herbaceous stratum: *Anthemis arvensis* (7.9%), *Lolium perenne* (7.5%), *Avena sterilis* (6.9%), *Phalaris minor* (6.4%), *Cirsium creticum ssp. triumfettii* (6.1%), *Rumex crispus* (5.6%), *Anagallis arvensis* (5.4%), *Trifolium alessandrinum* (4.2%), *Cichorium intybus* (3.8%) and *Vicia sativa* (3.8%). The herbaceous stratum in Site B was composed of fifty-three plant species, and their relative percentages ranged from 0.3 to 12.8%. More than half (50.4%) of the identified species was represented by the eight following *taxa*: *Picris echioides* (12.8%), *Picris hieracioides* (10.4%), *Senecio vulgaris* (5.87%), *Soncus oleraceus* (5.9%), *Equisetum arvense* (4.3%) and, with the same incidence (3.7%), *Medicago truncatula*, *Poa pratense* and *Trifolium repens*. The presence of some of the aforementioned *taxa* (e.g., *Cynodon dactilon*, *Daucus carota*, *Lolium perenne*, *Picris hieracioides*) has been also recorded in some semi-natural landscapes of Basilicata region (Freschi et al., 2015a). Moreover, among the *taxa* observed in the present study there were some species (e.g., *Lolium spp*., *Trifolium spp*., *Daucus carota*, *Convolvulus arvensis*, etc.) identified as components of the pasture available for organic Ancona laying hens, as well as of their diet (Mugnai et al., 2009).

After hen grazing, thirty *taxa* were observed in the site A, with a relative percentage ranging from 0.3 to 9.5% (Table 1). Among the most observed plant species there were *Anthemis arvensis* (9.51%), *Avena sterilis* (9.5%), *Cichorium intybus* (8.5%), *Cirsium creticum ssp. triumfettii* (8.2%), *Lolium perenne* (8.2%), *Polygonum aviculare* (7.9%); altogether, these six *taxa* accounted for 51.8% of the all observed species. Concerning Site B, there were forty-two plant species in the ground cover after hen grazing; seven of them accounted for more than half (54.5%) of the total identified *taxa*: *Picris echioides* (14.1%), *Picris hieracioides* (11.3%), *Trifolium repens* (6.3%), *Lactuca saligna* (6.1%), *Verbena officinalis* (6.1%), *Equisetum arvense* (5.5%), *Anagallis arvensis* (5.2%). Previous studies have shown that some of the plant species observed after hen grazing in both sites are particularly appetizing to hens (Mugnai et al., 2009; Horsted et al., 2006; Skřivan et al., 2015): in particular, Horsted et al. (2006) found, by the use of microhistological analysis of faeces, that *Lolium spp*., *Trifolium spp*. and *Polygonum spp.* were components of the diet of free-range laying hens. Other studies (Liu et al., 2011; Meng et al., 2016; Zheng et al., 2019) highlighted the importance of *Cichorium intybus* as a beneficial feed ingredient for poultry: for instance, Zheng et al. (2019) reported that, thanks to its healthy and nutritional properties, chicory can have positive effects on growth performance, carcass characteristics, meat and egg quality, and intestinal microbiota.

3.2 Biodiversity analysis in each site before and after hen grazing

Table 2 shows the results of alpha diversity analysis computed in Site A and Site B before and after hen grazing. The Student *t*-test revealed a significant difference in species richness and diversity between the two vegetation sampling periods in Site A. The value of Margalef's index (D) was significantly higher before than after hen grazing (3.56 vs. 2.75; p<0.001). Similarly, the value of the Shannon diversity index (H) was maximum at the beginning of the trial (2.13 vs. 1.73; p<0.001). Conversely, the value of species evenness (E) did not change significantly between the vegetation sampling periods. These results are probably due to the fact that some *taxa* which were previously identified as components of the herbaceous stratum of site A, were less observed (e.g., *Convolvulus arvensis, Cynodon dactilon, Medicago spinosa*, *Papaver rhoeas*) or no more observed (e.g., *Beta vulgaris*, *Calamintha nepeta*, *Cardaria draba*) after hen grazing. In nineteen *taxa* (e.g., *Amarantus retroflexus*, *Daucus carota*, *Lolium perenne*, *Lolium multifolium*), instead, the relative percentage was higher at the end than at beginning of the trial). The number of shared species between the two sampling periods was quite high (n=28, Table 1) with a high degree of overlap (C_S=0.74) as measured by the Sørensen index of similarity (Figure 1). Concerning Site B, the Student *t*-test revealed no significant differences in richness, diversity and evenness between the two periods (Table 2). Notwithstanding there was a decrease in the number (from 53 to 42; Table 1) of some *taxa* (e.g., *Vicia spp*., *Poligonum avicularis*, *Poa annua*), as well as a change in relative percentages of other plant species (e.g., *Trifolium repens*, *Lactuca saligna*, *Menta suaveolens*), we observed a high overlap between the two sampling periods (C_S= 0.78, Figure 1) with thirty-seven shared *taxa* (Table 1).

Table 1 Relative percentage (%) of plant species identified in Site A and B before and after hen grazing (*Note: Table continues on the next page*)

Taxa	Site A		Site B	
	Before grazing	After grazing	Before grazing	After grazing
Ajuga reptans	-	-	0.53	0.83
Amaranthus retroflexus	0.84	0.98	-	-
Amni majus	0.63	3.61	-	-
Anagallis arvensis	-	-	3.47	5.23
Anthemis arvensis	7.93	9.51	0.27	0
Apium nodiflorum	-	-	0.27	0.28
Arum italicum	-	-	0.27	0
Avena sativa	-	-	1.33	0.83
Avena sterilis	6.89	9.51	-	-
Beta vulgaris	2.71	0	-	-
Bromus erectus	-	-	2.4	1.1
Calamintha nepeta	0.63	0	-	-
Capsella bursa pastoris	-	-	0.27	0.55
Cerastium arvensis	-	-	1.33	0
Cichorium intybus	3.76	8.52	2.4	0.55
Cirsium creticum ssp. triumfettii	6.05	8.2	0.53	2.48
Convolvulus arvensis	2.71	1.31	0.8	1.38
Cynodon dactilon	2.51	1.31	-	-
Daucus carota	0.42	0.66	2.67	2.2
Dipsacus fullonum	-	-	0.27	0.55
Elymus caninum	0.42	0	-	-
Equisetum arvense	-	-	4.27	5.51
Erigeron canadensis	1.88	2.95	0.8	0.55
Eritrea centauris	-	-	0.27	0.83
Geranium dissectum	1.46	0	2.93	0.83
Geranium philicifolium	-	-	1.07	0
Geranium rotundifolia	-	-	0	0.55
Helminthotheca echioides	-	-	12.8	14.05
Holcus lanatus	-	-	0.53	0
Hordeum murinum	-	-	0.27	0
Hypericum perforatum	-	-	0.27	0
Kickxia elatine	1.46	3.93	-	-
Lactuca serriola	0.63	0	2.4	6.06
Lathyrus pratensis	0.42	0	0.27	0
Legousia speculum veneris	0.42	0.66	-	-
Lepidium draba	0.84	0	-	-
Linum multiflorum	-	-	0	0.28
Lythrum salicaria	-	-	0.27	0
Lolium multiflorum	2.09	4.26	-	-
Lolium perenne	7.52	8.2	2.67	4.68
Lumex criscus	-	-	0	1.38
Lysimachia arvensis	5.43	5.9	-	-
Malva silvestris	0.21	0	0.27	0
Matricaria romana	-	-	0	0.28
Medicago hybridus	-	-	1.6	3.31
Medicago lupulina	1.25	0	-	-
Medicago truncatula	0.63	0.33	3.73	2.2

Mentha longifolia	-	-	1.07	3.03
Mentha suaveolens	-	-	1.33	0.55
Onobrychis viciifolia	1.04	0	-	-
Onopordon acanthium	0.84	0.98	-	-
Papaver rhoeas	2.09	0.33	1.07	0.28
Petasites hybridus	-	-	0.27	0
Phalaris minor	6.47	2.95	0	0.28
Phleum nodosum	2.51	0	0.53	0.55
Picris echioides	2.3	3.28	-	-
Picris hieracioides	0.42	0.66	10.4	11.29
Plantago maior	-	-	0.8	1.93
Poa annua	-	-	1.87	0
Poa pratense	-	-	3.73	2.48
Policarpon teraphyllum ssp. diphyllum	0.42	0	-	-
Polycarpon tetraphyllum	0.42	1.31	-	-
Polygonum aviculare	2.92	7.87	0.27	0
Potentilla reptans	-	-	2.13	1.1
Ranunculus bulbosus	0.84	0.33	-	-
Rubus ulmifolius	-	-	0.27	0.28
Rumex crispus	5.64	5.57	1.33	0.55
Scabiosa maritima	0	0.33	-	-
Scandix pecten veneris	0.84	0	-	-
Senecio vulgaris	-	-	5.87	3.03
Setaria italica	0.42	0	-	-
Sherardia arvensis	0.21	0	-	-
Sisymbrium orientale	3.13	0.33	-	-
Smirnum polisacrum	0.21	0	-	-
Sonchus oleraceus	-	-	5.87	3.86
Soncus arvensis	1.46	1.31	-	-
Trifolium alessandrinum	4.18	3.61	-	-
Trifolium campestre	0.21	0	0.8	0
Trifolium pratense	-	-	3.47	0.55
Trifolium repens	-	-	3.73	6.34
Verbena officinalis	-	-	2.4	6.06
Veronica anagallis aquatica	-	-	0.27	0.28
Veronica arvensis	-	-	0.8	1.1
Vicia cracca	-	-	0.27	0
Vicia sativa	3.76	1.31	0.27	0
Total	100	100	100	100

Table 2 Measures of alpha (mean ± SD) diversity analysis computed in each site before and after hen grazing

Index	Site A			Site B		
	Before grazing	After grazing	*p-value*	Before grazing	After grazing	*p-value*
Species richness (D)	3.56 ± 0.74	2.75 ± 0.57	0.001	2.99 ± 0.61	3.04 ± 0.69	NS
Species diversity (H)	2.13 ± 0.33	1.73 ± 0.35	0.001	1.85 ± 0.34	1.87 ± 0.37	NS
Species evenness (E)	1 ± 0	1 ± 0	NS	1 ± 0	1 ± 0	NS

NS = not significant

Table 3 Measures of alpha (mean ± SD) diversity analysis computed between sites before and after hen grazing

Index	Before grazing			After grazing		
	Site A	Site B	*p-value*	Site A	Site B	*p-value*
Species richness (D)	3.56 ± 0.74	2.99 ± 0.61	0.001	2.75 ± 0.57	3.04 ± 0.69	0.05
Species diversity (H)	2.13 ± 0.33	1.85 ± 0.34	0.001	1.73 ± 0.35	1.87 ± 0.37	0.05
Species evenness (E)	1 ± 0	1 ± 0	NS	1 ± 0	1 ± 0	NS

NS = not significant

3.3 Biodiversity analysis between sites before and after hen grazing

As shown in Table 3, there were significant differences ($p<0.001$) in species richness and diversity between the sites at the beginning of the trial, with the ground cover of site A being richer and more diverse compared to that of Site B. The observed differences between the sites seem to be corroborated by the low number of shared species (n=18, Table 1) and the consequent low overlap (C_S= 0.36) obtained by computing by the Sørensen index of similarity (Table 4). After hen grazing, the values of species richness and diversity were significantly higher in Site B than in A ($p<0.05$; Table 3). There was also a decrease of the number of shared species (n=11, Table 1) and of the value of Sørensen index of similarity (C_S= 0.30, Table 4).

Table 4 Values of the Sørensen index for similarity (C_S) computed in each site and between sites before and after hen grazing

Similarity index	Comparisons			
	In each site		Between sites	
	A	B	Before grazing	After grazing
Sørensen (C_S)	0.74	0.78	0.36	0.30

4 Conclusions

Agroforestry presupposes a rational integration and management of trees, crops and livestock on the same plot of land for the mutual benefit of all components (Nair, 1993; Burgess et al., 2015). It is from this perspective that the present study, aimed at evaluating the impact of poultry integrated into hazelnut orchards, was carried out. Two sites were chosen in this regard: they were different for plant species presence and distribution of their ground cover, with one of them being richer and more diverse compared to the other site. Our findings showed that the use of hens influenced quantitatively and qualitatively the herbaceous stratum of the study sites: the number of *taxa* composing the ground cover decreased in both sites (Site A: from 46 to 30; Site B: from 53 to 42), whereas the relative frequencies varied (increased or decreased) according to the *taxon*. Such variation in vegetation composition was highlighted by computing different measures of alpha diversity, which revealed that, after hen grazing, species richness and diversity were lower in site A and higher in site B, although the latter difference was not statistically significant. When comparing the sites, we found that species richness and diversity were significant higher in site A than in site B at the beginning of the trial; however, hen grazing made the ground cover assemblage of site B richer and more diverse compared to that of site A. These results seem to indicate that the grazing of hens into an orchard can reduce growth of the ground cover, rather than impacting on its biodiversity, as confirmed by the value of the Sørensen index. These results reflect those of Liu et al. (2013) who observed the maintenance of similar aboveground plant biomass under chicken grazing and the unstocked control. Overall, our findings, although limited because of the small number of study sites and the duration of the research, suggest that hens can be positively integrated into hazelnut orchards. However, further research might explore other aspects (differential grazing to variable plant phenology, soil fertility, dietary preferences) to fully understand the implications of poultry grazing in a such agroforestry system.

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