

# Growth control of herbaceous ground cover and egg quality from an integrated poultry-hazelnut orchard system

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## Abstract

The present study was designed to assess the impact of hen grazing on plant biodiversity in the ground cover of hazelnut orchards, as well as the quality of eggs from hens grazing in the orchards. Two different sites of hazelnut orchards located in Basilicata region were selected to conduct this study. The results showed that hen grazing affected the composition of herbaceous stratum of the orchards. By using the quadrat method, a total of 99 (46 in site A and 53 in site B) plant species were identified at the beginning of the trial. After hen grazing, the number of identified plants dropped to 72 (30 in site A and 42 in site B). Amongst the most consumed species we found *Lolium sp.*, *Trifolium sp.*, *Polygonum sp.*, *Cichorium intybus*, *Cynodon dactylon*. Alpha diversity analysis (species richness, diversity and evenness) as well as beta diversity analysis (Morisita-Horn and Sørensen indices) confirmed that hen grazing played a role in controlling vegetation growth. Concerning egg quality, most of the chemical parameters differed significantly when comparing the eggs from the sites with those from the control group, indicating that egg quality was influenced by the food resources available in each site. Overall, our results confirm that poultry may play a positive role in an orchard system.

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## Introduction

In recent years, after restricting and, in some cases, banning the use of conventional intensive systems, there has been a renewed interest in pasture-based systems and pasture-raised livestock products. This is particularly true for poultry, since the use of pasture-based systems for poultry production is growing due to consumer demand for speciality *natural* and *welfare-friendly* meat and eggs (Antell and Cizuk, 2006; Anderson, 2011; Sossidou *et al.*, 2011; Chiello *et al.*, 2016). These concerns have led to a considerable amount of research on comparing conventional raising systems with range raising systems, as well as on the effects of each system on egg quality (Krawczyk, 2009, 2011; Matt *et al.*, 2009; Karsten *et al.*, 2010; Anderson, 2011; Holt *et al.*, 2011; Küçükyılmaz *et al.*, 2012; Rizzi and Marangon, 2012). Other studies have focused on forage consumption of laying hens on free range (Antell and Cizuk, 2006; Horsted *et al.*, 2007; Singh and Cowieson, 2013; Skřivan *et al.*, 2015). *Lolium sp.*, *Trifolium sp.*, *Polygonum sp.*, *Cichorium intybus*, *Cynodon dactylon* were found to be, amongst others, the most occurring plant species in grazing hens' diet (Horsted *et al.*, 2006; Mugnai *et al.*, 2009; Liu *et al.*, 2011; Skřivan *et al.*, 2015; Meng *et al.*, 2016; Sirri *et al.*, 2018; Zheng *et al.*, 2019).

Free-range system is a recognizing animal welfare friendly method, as it provides birds with sunlight, fresh air, ample space, nutrients, *etc.*, as well as allows them to express natural behaviours (Berg, 2002; Sossidou *et al.*, 2011; Mohammed *et al.*, 2013). Moreover, raising poultry on free-range offers many advantages in terms of farm soil fertility, disease prevention, weed control, farm diversity, environmental sustainability, farm profitability, *etc.* (Glatz *et al.*, 2005; Sossidou *et al.*, 2011; Liu *et al.*, 2013).

An interesting approach is to integrate hen grazing within high value fruit trees systems (*e.g.*, olive, hazelnut, walnut, almond, chestnut, apple, and pear systems) in order to obtain an additional source of income while providing weed control and fertilisation, thus lowering costs and impact of the management. Previously published studies on the association between hen grazing and orchards (Clark and Gage, 1996; Lavigne *et al.*, 2012; Paolotti *et al.*, 2016; Rosati *et al.*, 2016; Timmermans, 2016) have only focused on fruit quality, weed and pest control, sustainability. In contrast, there is much less information about the effects of an integrated poultry-orchard system on herbaceous plant species diversity or egg quality. Therefore, the present study was designed to investigate: i) the impact of hen grazing integrated into hazelnut orchards on plant biodiversity in the ground cover; ii) the quality of eggs from hens grazing in the orchards.

## Materials and methods

### Study sites

The present study - which is part of a project called 'CORILUS - Sustainable Lucanian Coriliculture' whose objective is to improve the hazelnut production in Basilicata region (south of Italy) - is a first attempt to analyse an integrated poultry-hazelnut orchard system. Within the aforementioned project, two pilot farms were selected according to the following criteria: i) farm location (Province of Potenza); ii) age of the trees (three years); iii) unproductive condition (trees require roughly 4 years to mature before they are able to provide hazelnuts); iv) cultivar (*Corylus avellana*, 'Tonda di Giffoni'); v) tree spacing (3×5 m).

The study was conducted from May 2019 to September 2019 in two different sites, one for each selected farm. 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 mean annual air temperature is +12°C. The hazelnut trees are here planted in a medium-textured soil tending to sandy (sand: 65.10%; clay: 21.50%; silt: 13.40%).

In the second site (site B; 40°24'16.76"N - 15°43'44.80"), which spans approximately 5 ha, the elevation is 660 m a.s.l., the mean annual precipitation and the mean annual air temperature are 780 mm and 13°C, respectively. A loamy textured soil (sand: 48.40%; clay: 23.20%; silt: 28.40%) was found in this site.

### Animals, housing and feeding

A total of 90 hens belonging to a commercial egg-laying genotype were purchased from a local poultry firm. At 25-weeks of age, the hens were randomly divided into three homogeneous groups of 30 birds each. Hens from group A and B were assigned to site A and B, respectively. Hens composing the third group (control group) were kept at the local poultry firm under free-range farming condition (2 m<sup>2</sup>/bird).

Each site was divided into 3 homogeneous areas, each provided with a mobile coop to confine the hens (5 birds/m<sup>2</sup>) for protection from predators during the night and to encourage egg-laying in coop nests. Feed and water were provided outside each mobile coop with feeders and automatic drinkers, respectively. All hen groups were fed on the same basal diet, a commercial feed with the following characteristics: crude protein 17%, crude fat 4.50%, crude fibre 4.50%, crude ash 13%, lysine 0.90%, methionine 0.36%, Ca 4%, P 0.56%, Na 0.17%, vitamin A 6000 IU, vitamin D3 1800 IU. Besides, the hens from both site groups were given access to the pasture for 12 hours a day (from 07:00 a.m. to 07:00 p.m.), with drinking water being available on an *ad libitum* basis.

### Ground cover composition assessment

The botanical composition of the herbaceous stratum of each site was evaluated before (first decade of May) and after hen grazing (first decade of September) by using the quadrat method (Bonham, 1989) modified by Rizzardini *et al.* (2019). Briefly, in each site 320 m 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<sup>2</sup>) arranged at a distance of 1 m from each other. The collected specimens were identified according to Flora d'Italia (Pignatti *et al.*, 2019) and follow the taxonomic nomenclature of this text.

### Chemical composition of eggs

In each site, eggs were daily gathered during the whole

experimental period. For chemical analysis, 11 eggs were randomly selected in each site from each mobile coop on the last 3 days of the experimental period. Following Karsten *et al.* (2010), 3 eggs were randomly selected from the collected 11 eggs, and the egg yolks were pooled. A total of 3 pooled-yolk samples per group (9 yolks total) were used to evaluate the following parameters: dry matter by oven drying the sample at 100°C for 18 h; total lipids (Folch *et al.*, 1957); protein and ash (AOAC, 2006); cholesterol (Meluzzi *et al.*, 1993); vitamin A, D and E (Karsten *et al.*, 2010); fatty (monounsaturated, polyunsaturated, and saturated) acids (Matt *et al.*, 2009); lysozyme (Labella *et al.*, 2016).

### Statistical analysis

For each site, data on the identified species before and after hen grazing were used to calculate the relative percentage (*rp*) of a *taxon* composing the ground cover (Freschi *et al.*, 2014, 2015a, 2016, 2017):

$$rp = (n/N) \times 100 \quad (1)$$

where: *n* is the number of occurrences attributed to a given *taxon* in a given period of the trial (before or after hen grazing); *N* is the total number of identified occurrences in that given period. The Mann-Whitney *U*-test was used to compare the changes in vegetation composition between the two sampling periods; the statistical significance was set at *P*<0.05.

Alpha diversity analysis was computed in each site by using the following indices: i) species richness (*D*), for which the higher the value the greater the richness (Margalef, 1958); ii) species diversity (*H*) (Shannon and Weaver, 1949), whose value usually ranges between 1.5 and 3.5 and often does not exceed 4 (Margalef, 1972); and iii) species evenness (*E*) (Buzas and Gibson, 1969), whose value ranges between 0 and 1, where 1 indicates that all the food items are used to an equal extent. Differences in richness, diversity, and evenness calculated before and after hen grazing were analysed by the Student *t*-test.

Two beta diversity indices were used in each site in order to measure the extent of differentiation in species composition between the two sampling periods: i) the quantitative Morisita-Horn index (*C<sub>MH</sub>*) (Morisita, 1959), which takes into account the species present in each sample and their abundance (Magurran, 2004); and ii) the qualitative Sørensen index (*C<sub>S</sub>*) (Sørensen, 1948), which only considers presence/absence data. Typically, the value of these indices ranges from 0 (complete dissimilarity between sites) to 1 (complete similarity between sites). *C<sub>MH</sub>* values were classified according to the scale proposed by Langton (1982): 0 < *C<sub>MH</sub>* ≤ 29, small overlap; 30 ≤ *C<sub>MH</sub>* ≤ 59, medium overlap; and *C<sub>MH</sub>* ≥ 60, high overlap.

In each site, the Student *t*-test was used to determine the significance of the differences in chemical composition of eggs from experimental group and those from control group.

## Results and discussion

### Composition of the cover vegetation on the ground

A total of forty-six plant species were identified in the herbaceous stratum of site A at the beginning of the trial (Table 1). Almost half of the *taxa* (46%) were observed in low percentages (<1%); among them, there were *Daucus carota*, *Picris hieracioides*, *Malva sylvestris*, *Trifolium campestre*, *etc.* Other *taxa* were instead

observed at higher percentages: for instance, the following ten *taxa* accounted for 57.63% of the all observed species composing the herbaceous stratum: *Anthemis arvensis* (7.93%), *Lolium perenne* (7.52%), *Avena sterilis* (6.89%), *Phalaris minor* (6.47%), *Cirsium creticum triumfettii* (6.05%), *Rumex crispus* (5.64%), *Lysimachia arvensis* (5.43%), *Trifolium alexandrinum* (4.18%), *C. intybus* (3.76%) and *Vicia sativa* (3.76%). Concerning the Site B, the first vegetation sampling allowed to identify fifty-three plant *taxa* composing the herbaceous stratum (Table 2), with relative percentages ranging from 0.27 to 12.80%. More than half (50.40%) of the identified species was represented by the eight following *taxa*: *Helminthotheca echioides* (12.8%), *P. hieracioides* (10.4), *Senecio vulgaris* (5.87), *Sonchus oleraceus* (5.87), *Equisetum arvense* (4.27), *Medicago truncatula* (3.73), *Poa pratense* (3.73), *T. repens* (3.73).

The composition of herbaceous vegetation, along with other farm characteristics (rainfall, temperature, altitude, etc.), are key elements differentiating the two study sites. The presence of some *taxa* (e.g., *C. dactylon*, *D. carota*, *L. perenne*, *P. hieracioides*) in the ground cover of the sites has been also recorded in some semi-natural landscapes of Basilicata region (Freschi *et al.*, 2015a, 2015b). Moreover, among the *taxa* observed in the present study, there were some species (e.g., *Lolium spp.*, *Trifolium spp.*, *D. 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 0.33 to 9.51% (Table 1). The most observed *taxa* were *Anthemis arvensis* (9.51%), *A. sterilis* (9.51%), *C. intybus* (8.52%), *C. creticum ssp. triumfettii* (8.20%), *L. perenne* (8.20%), *Polygonum aviculare* (7.87%); altogether, these six *taxa* accounted for 51.81% of the all observed species. The plant species observed in site B at the end of the trial were forty-two (Table 2), with seven of them accounting for more than half (54.54%) of the total identified *taxa*: *H. echioides* (14.05%), *P. hieracioides* (11.29%), *T. repens* (6.34%), *Lactuca serriola* (6.06%), *Verbena officinalis* (6.06%), *E. arvense* (5.51%), *L. arvensis* (5.23%). Previous studies have shown that, some of the plant species observed after hen grazing in both sites are particularly appetizing to hens (Horsted *et al.*, 2006; Mugnai *et al.*, 2009; Skřivan *et al.*, 2015; Sirri *et al.*, 2018): in particular, Horsted *et al.* (2006) found, by the use of microhistological analysis of faeces, that *Lolium sp.*, *Trifolium sp.*, *Polygonum sp.* 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 *C. intybus* as a beneficial feed ingredient for poultry.

Changes observed in the herbaceous stratum composition of site A and B between the two vegetation sampling periods are depicted in Figures 1 and 2, respectively.

The Mann-Whitney *U* test revealed a significant difference ( $P < 0.001$ ) between the two vegetation sampling periods in site A, thus indicating that the hens played an important role on controlling vegetation growth. This result is probably due to the fact that some *taxa* which were previously identified as components of the herbaceous stratum of the site, were less observed (e.g., *C. arvensis*, *C. dactylon*, *M. truncatula*, *Papaver rhoeas*, etc.) or no more observed (e.g., *Elymus caninum*, *Beta vulgaris ssp. maritima*, *Calamintha nepeta ssp. nepeta*, *Lepidium draba*, etc.) after hen grazing. For nineteen *taxa*, instead, there was an increase of their presence: the relative percentage of *Amaranthus retroflexus*, *D. carota*, *L. perenne*, *L. multiflorum*, etc. was higher at the end than at beginning of the trial. With regard to site B (Figure 2), the difference observed between the sampling periods was not

significant, although there was a decrease in the number (from 53 to 42) of some *taxa* (e.g., *Vicia sp.*, *Polygonum aviculare*, *P. annua*, etc.), as well as a change in relative percentages of other *taxa* (e.g., *T. repens*, *L. serriola*, *Mentha suaveolens*, etc.).

**Table 1. Relative percentage (%) of plant species identified in Site A before and after hen grazing.**

Species	Before grazing	After grazing
<i>Elymus caninum</i>	0.42	0.00
<i>Amaranthus retroflexus</i>	0.84	0.98
<i>Amni majus</i>	0.63	3.61
<i>Lysimachia arvensis</i>	5.43	5.90
<i>Anthemis arvensis</i>	7.93	9.51
<i>Avena sterilis</i>	6.89	9.51
<i>Beta vulgaris ssp. maritima</i>	2.71	0.00
<i>Calamintha nepeta ssp. nepeta</i>	0.63	0.00
<i>Lepidium draba</i>	0.84	0.00
<i>Cichorium intybus</i>	3.76	8.52
<i>Cirsium creticum ssp. triumfettii</i>	6.05	8.20
<i>Convolvulus arvensis</i>	2.71	1.31
<i>Cynodon dactylon</i>	2.51	1.31
<i>Daucus carota</i>	0.42	0.66
<i>Erigeron canadensis</i>	1.88	2.95
<i>Geranium dissectum</i>	1.46	0.00
<i>Polycarpon tetraphyllum ssp. diphylum</i>	0.42	1.31
<i>Kickxia elatine ssp. elatine</i>	1.46	3.93
<i>Lactuca serriola</i>	0.63	0.00
<i>Lathyrus pratensis</i>	0.42	0.00
<i>Legousia speculum veneris</i>	0.42	0.66
<i>Lolium multiflorum</i>	2.09	4.26
<i>Lolium perenne</i>	7.52	8.20
<i>Malva sylvestris</i>	0.21	0.00
<i>Medicago lupulina</i>	1.25	0.00
<i>Medicago truncatula</i>	0.63	0.33
<i>Onobrychis viciifolia</i>	1.04	0.00
<i>Onopordon acanthium</i>	0.84	0.98
<i>Papaver rhoeas</i>	2.09	0.33
<i>Phalaris minor</i>	6.47	2.95
<i>Phleum nodosum</i>	2.51	0.00
<i>Picris echioides</i>	2.30	3.28
<i>Picris hieracioides</i>	0.42	0.66
<i>Polycarpon teraf.</i>	0.42	0.00
<i>Polygonum aviculare</i>	2.92	7.87
<i>Ranunculus bulbosus</i>	0.84	0.33
<i>Rumex crispus</i>	5.64	5.57
<i>Scabiosa maritima</i>	0.00	0.33
<i>Scandix pecten veneris</i>	0.84	0.00
<i>Setaria italica</i>	0.42	0.00
<i>Sherardia arvensis</i>	0.21	0.00
<i>Sisymbrium orientale</i>	3.13	0.33
<i>Smimum polisacrum</i>	0.21	0.00
<i>Soncus arvensis</i>	1.46	1.31
<i>Trifolium alexandrinum</i>	4.18	3.61
<i>Trifolium campestre</i>	0.21	0.00
<i>Vicia sativa</i>	3.76	1.31
	100	100

## Alpha and beta diversity analysis

The results of alpha diversity analysis conducted in site A are shown in Table 3. The Student *t*-test revealed a significant difference in species richness and diversity between the two vegetation sampling periods. The value of Margalef's index (*D*) was significantly higher before than after hen grazing (3.33 vs 2.75;  $P < 0.001$ ). Similarly, the value of the Shannon diversity index (*H*) was maximum at the beginning of the trial (1.99 vs 1.73;  $P < 0.01$ ).

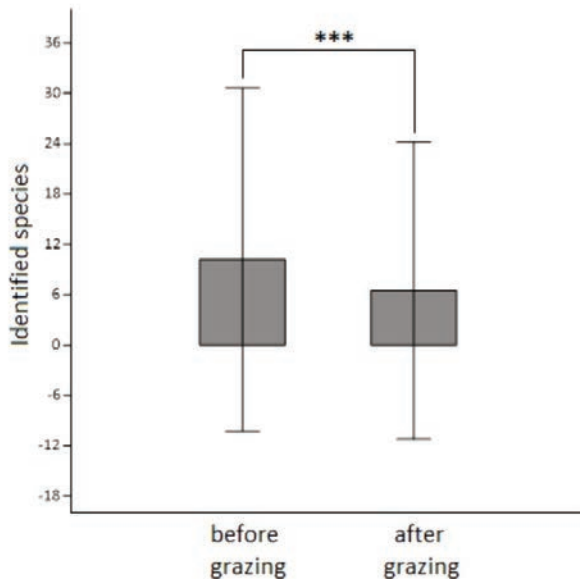


Figure 1. Changes in vegetation composition of Site A due to hen grazing. The bars represent the mean values of plant species identified by using the quadrat method, whereas the whiskers represent 95% confidence intervals (\*\* $P < 0.001$ ).

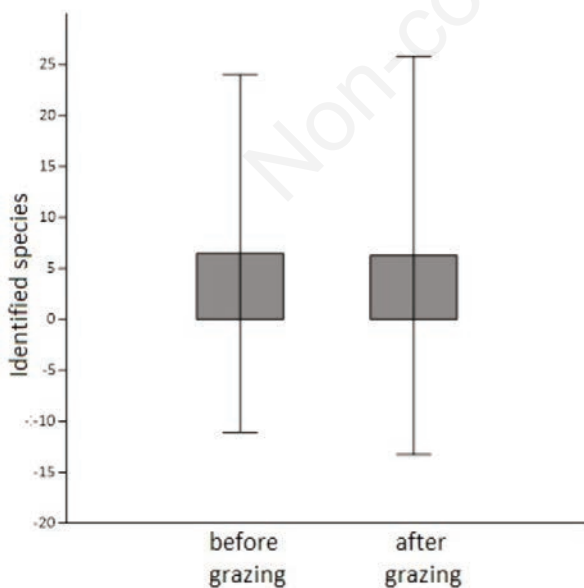


Figure 2. Changes in vegetation composition of Site B due to hen grazing. The bars represent the mean values of plant species identified by using the quadrat method, whereas the whiskers represent 95% confidence intervals.

Table 2. Relative percentage (%) of plant species identified in Site B before and after hen grazing.

Species	Before grazing	After grazing
<i>Ajuga reptans</i>	0.53	0.83
<i>Anagallis arvensis</i>	3.47	5.23
<i>Anthemis arvensis</i>	0.27	0.00
<i>Apium nodiflorum</i>	0.27	0.28
<i>Arum italicum</i>	0.27	0.00
<i>Avena sativa</i>	1.33	0.83
<i>Bromus erectus</i>	2.40	1.10
<i>Capsella bursa pastoris</i>	0.27	0.55
<i>Cerastium arvensis</i>	1.33	0.00
<i>Cichorium intybus</i>	2.40	0.55
<i>Cirsium triumfetti</i>	0.53	2.48
<i>Convolvulus arvensis</i>	0.80	1.38
<i>Daucus carota</i>	2.67	2.20
<i>Equisetum arvense</i>	4.27	5.51
<i>Erigeron canadensis</i>	0.80	0.55
<i>Eritrea centauris</i>	0.27	0.83
<i>Geranium dissectum</i>	2.93	0.83
<i>Geranium philicifolium</i>	1.07	0.00
<i>Geranium rotundifolia</i>	0.00	0.55
<i>Holcus lanatus</i>	0.53	0.00
<i>Hordeum murinum</i>	0.27	0.00
<i>Hypericum perforatum</i>	0.27	0.00
<i>Lactuca serriola</i>	2.40	6.06
<i>Lathyrus pratensis</i>	0.27	0.00
<i>Linum multiflorum</i>	0.00	0.28
<i>Lythrum salicaria</i>	0.27	0.00
<i>Lolium perenne</i>	2.67	4.68
<i>Lumex criscus</i>	0.00	1.38
<i>Malva silvestris</i>	0.27	0.00
<i>Matricaria romana</i>	0.00	0.28
<i>Medicago hybridus</i>	1.60	3.31
<i>Medicago truncatula</i>	3.73	2.20
<i>Mentha longifolia</i>	1.07	3.03
<i>Mentha suaveolens</i>	1.33	0.55
<i>Papaver rhoeas</i>	1.07	0.28
<i>Petasites hybridus</i>	0.27	0.00
<i>Phalaris minor</i>	0.00	0.28
<i>Phleum nodosum</i>	0.53	0.55
<i>Helminthotheca echioides</i>	12.80	14.05
<i>Picris hieracioides</i>	10.40	11.29
<i>Plantago maior</i>	0.80	1.93
<i>Poa annua</i>	1.87	0.00
<i>Poa pratense</i>	3.73	2.48
<i>Polygonum aviculare</i>	0.27	0.00
<i>Potentilla reptans</i>	2.13	1.10
<i>Rubus ulmifolius</i>	0.27	0.28
<i>Rumex crispus</i>	1.33	0.55
<i>Senecio vulgaris</i>	5.87	3.03
<i>Sonchus oleraceus</i>	5.87	3.86
<i>Trifolium campestre</i>	0.80	0.00
<i>Trifolium pratense</i>	3.47	0.55
<i>Trifolium repens</i>	3.73	6.34
<i>Verbena officinalis</i>	2.40	6.06
<i>Veronica anagallis aquatica</i>	0.27	0.28
<i>Veronica arvensis</i>	0.80	1.10
<i>Vicia cracca</i>	0.27	0.00
<i>Vicia sativa</i>	0.27	0.00
<i>Dipsacus fullonum</i>	0.27	0.55
	100	100

**Table 3. Measures of alpha (mean±SD) diversity analysis in each site computed before and after hen grazing.**

Index	Site A		Sign.	Site B		Sign.
	BF	AF		BF	AF	
Species richness ( <i>D</i> )	3.33±0.15	2.75±0.08	***	2.99±0.08	3.04±0.09	NS
Species diversity ( <i>H</i> )	1.99±0.08	1.73±0.05	**	1.85±0.04	1.87±0.05	NS
Species evenness ( <i>E</i> )	0.94±0.03	1±0	NS	1±0	1±0	NS

BF, before grazing; AG, after grazing; Sign., significance. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; NS, not significant.

These results are mainly due to the aforementioned decrease in the number of *taxa* composing the herbaceous stratum of the site (from 46 to 30) and, to a lesser extent, to the change in the value of the relative percentage of some species. Conversely, the value of species evenness (*E*) did not change significantly between the vegetation sampling periods. Concerning beta diversity analysis (Table 4), the value of the Morisita-Horn quantitative index ( $C_{MH}$ ) in site A was 0.874, indicating that the degree of overlap between the two sampling periods was high. The Sørensen qualitative similarity index ( $C_s$ ) was 0.756, as consequence of the high number of plant species shared by the two periods. Concerning site B, according the Student *t*-test, there were no significant differences in richness, diversity and evenness between the two periods (Table 3): these results seem to confirm that the control of turf growth by the hens was less severe than it was in site A. This seems to be also corroborated by both the Morisita-Horn and Sørensen indices: both the values ( $C_{MH} = 0.903$ ;  $C_s = 0.778$ ; Table 4) indicated a high overlap between the two vegetation sampling periods, and were also higher than those observed in site A. Overall, our results reflect those of Liu *et al.* (2013) who observed the maintenance of similar aboveground plant biomass under chicken grazing and the unstocked control.

### Egg composition

The chemical composition of eggs from site A (group A) and B (group B) is shown in Tables 5 and 6, respectively. It is now well established from a variety of studies (Roberts, 2004; Ahmadi and Rahimi, 2011; Yang *et al.*, 2014) that egg composition can be affected by several factors (*e.g.*, age, breed, nutrition, type of rearing system, time of oviposition, *etc.*), which make direct comparison of results from different investigations difficult. In the present study, the values of some chemical parameters were broadly in line with previous studies (Basmacioğlu and Ergül, 2005; Matt *et al.*, 2009; Krawczyk *et al.*, 2009, 2011; Anderson, 2011; Küçükylmaz *et al.*, 2012; Rizzi and Marangon, 2012). For example, there were similarities between the protein content of eggs from both sites and that reported by Krawczyk (2009) for backyard hens or Matt *et al.* (2009) and Küçükylmaz *et al.* (2012) for caged hens. In contrast, our values were lower than those found in a study on the quality of organic eggs from hybrid and Italian breed hens (Rizzi and Marangon, 2012).

As shown in Tables 5 and 6, the Student *t*-test showed significant differences between the eggs from both sites (group A and B) and those from control group for most of the chemical parameters. For example, eggs from group A and B had significant ( $P < 0.001$ ) lower carbohydrate, lipid and protein content than those from control group. The difference in cholesterol content between the eggs from both sites and those from control group was also statistically significant ( $P < 0.001$ ): compared to control eggs, those from group A were richer in cholesterol, whereas eggs from group B were lower. Concerning fatty acid composition, the contents of saturated and polyunsaturated fatty acids were in significant higher

**Table 4. Measures of beta diversity analysis in each site computed before and after hen grazing.**

Index	Site A	Site B
Morisita-Horn (CMH)	0.874	0.903
Sorensen (CS)	0.756	0.778

**Table 5. Chemical composition of eggs laid by hens grazing in site A (mean±SD).**

Parameter	Group A	Control group	Sign.
Carbohydrates, %	0.70±0.25	1.01±0.24	***
Proteins, %	12.41±0.51	13.01±0.52	***
Lipids, %	9.50±0.51	11.11±0.52	***
Dry matter, %	18.74±0.51	18.72±0.49	NS
Ash, %	1.76±0.05	1.11±0.05	***
Monounsaturated fatty acids, %	4.38±0.05	6.87±0.05	***
Polyunsaturated fatty acids, %	3.29±0.03	2.76±0.05	***
Saturated fatty acids, %	6.38±0.04	5.39±0.04	***
Cholesterol, mg/g	353.58±3.37	322.91±3.11	***
Vitamin A, mg 100 g <sup>-1</sup>	0.32±0.04	0.28±0.05	**
Vitamin E, mg 100 g <sup>-1</sup>	5.40±0.04	5.48±0.05	***
Vitamin D, mg 100 g <sup>-1</sup>	0.60±0.08	1.80±0.25	**
Lysozyme, %	0.50±0.07	0.49±0.06	NS

Sign., significance. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; NS, not significant.

**Table 6. Chemical composition of eggs laid by hens grazing in site B (mean±SD).**

Parameter	Group B	Control group	Sign.
Carbohydrates, %	0.71±0.25	1.01±0.24	***
Proteins, %	12.56±0.51	13.01±0.52	***
Lipids, %	8.71± 0.52	11.11±0.52	***
Dry matter, %	18.26±0.48	18.72±0.49	***
Ash, %	1.60±0.05	1.11±0.05	***
Monounsaturated fatty acids, %	8.27±0.05	6.87±0.05	***
Polyunsaturated fatty acids, %	3.07±0.04	2.76±0.04	***
Saturated fatty acids, %	9.86±0.03	5.39±0.04	***
Cholesterol, mg/g	295.58±3.37	322.91±3.11	***
Vitamin A, mg 100 g <sup>-1</sup>	0.23±0.04	0.28±0.05	**
Vitamin E, mg 100 g <sup>-1</sup>	5.42±0.04	5.48±0.05	***
Vitamin D, mg 100 g <sup>-1</sup>	1.00±0.09	1.80±0.25	***
Lysozyme, %	0.48±0.05	0.49±0.06	NS

Sign., significance. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; NS, not significant.

amount ( $P < 0.001$ ) in eggs from group A than in control eggs, which were instead characterised by the highest content of monounsaturated fatty acids. Results from site B showed that, the eggs from group B had the highest content of saturated, monounsaturated, and polyunsaturated fatty acids. With regard to vitamin content, compared with control hens, group A individuals produced eggs with the highest level of vitamin A and the lowest of vitamin E and D. Eggs from group B were characterised by the highest content of the aforementioned vitamins. There were no significant differences in lysozyme content between the eggs from both experimental groups and those from control group.

Studies correlating nutrition and rearing system with some chemical parameters of eggs have generally produced conflicting results. For instance, previous studies (Minelli *et al.*, 2007; Matt *et al.*, 2009) have shown that the cholesterol content was greater in organic eggs than in conventional ones. Conversely, Krawczyk *et al.* (2011) found a lower cholesterol content in eggs from free-range system. Other studies (Karsten *et al.*, 2010; Anderson, 2011; Küçükylmaz *et al.*, 2012) found no difference in cholesterol content between the range- and cage-produced eggs. The same considerations apply with respect to the vitamin content of eggs recorded in the present study: in fact, Krawczyk *et al.* (2011) found a higher vitamin A concentration in free-range eggs, whereas Matt *et al.* (2009) reported that the concentration vitamin A was higher in conventional eggs. This corroborates the idea that, irrespective of management system, the vitamin content of eggs depends on the vitamin content of feed (Melluzzi *et al.*, 2000; Leeson and Caston, 2003).

Based on the above evidences and given that the hens received the same feed, it can be stated that the observed differences between eggs from the two sites are mainly related to the variation in hen diet, depending on the local availability and abundance of different food resources in each site. This seems to be in accordance with previous research (Mohammed *et al.*, 2013) which showed that, the crop content of the outdoor hens consisted, among others, of plant material, grit stones, snails and oyster shells, seeds, insects, worms, and larvae. After all, it has been suggested that fat content in free-range eggs can be increased by the consumption of forage, wild seeds and insects (Anderson, 2011). Krawczyk *et al.* (2011) reported that eating fresh plants and herbs from the free-range led to low egg levels of lipids and total cholesterol. According to the same authors, the increased levels of protein of free-range eggs associated with invertebrates and plants enriching the diets consumed by the hens. Similarly, insects, worms, and larvae, along with plant species composing the ground vegetation of each site, may also have played a role in the difference observed in egg composition.

## Conclusions

It is well established that allowing hens to free-range in a pasture offer several advantages in terms of soil fertility, weed control, farm profitability, *etc.*. Integrating poultry into an orchard may offer the same advantages, thus resulting a promising tool for developing more sustainable farming systems. It is from this perspective that the present study, aimed at evaluating the impact of poultry integrated into hazelnut orchard, was carried out in two different sites. Our findings showed that the use of hens influenced the herbaceous stratum of the study sites; this variation in vegetation composition was measured by computing some univariate measures of alpha diversity. After grazing, species richness and diversity were lower in site A and higher in site B, but with the latter difference

not being statistically significant. However, the observed values indicated more a control of vegetation growth by hens grazing than an impact on it, as confirmed by beta diversity analysis.

Concerning egg quality, compared to control eggs, those from both sites had significantly lower contents of carbohydrates, proteins, lipids, vitamin E and D. There were differences in fatty acids and vitamin A content: eggs from site A had a higher content of saturated and monounsaturated fatty acids and a higher content of vitamin A than control eggs. Eggs from site B were instead characterised by the highest content of saturated, monounsaturated and polyunsaturated fatty acids and the lowest content in vitamin A. These results seem to indicate that egg quality was influenced by the food resources (*e.g.*, plants, wild seeds and insects) available in each site.

Overall, our findings, although limited because the study sites are different and not comparable, suggest that hens can be positively integrated into hazelnut orchards. However, further research might explore other aspects (soil fertility, dietary preferences, *etc.*) to fully understand the implications of poultry grazing in an orchard system.

## Highlights

- The impact of an integrated poultry-hazelnut orchard system on herbaceous plant species diversity and on egg quality was evaluated.
- Hen grazing may benefit herbaceous ground cover biodiversity of the orchards.
- Egg quality may be affected by the food resources available in the orchards.

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