# Cover crops for managing weeds, soil chemical fertility and nutritional status of organically grown orange orchard in Sicily

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## Abstract

Cover crops can offer significant advantages in the agronomic management of citrus orchards in Mediterranean environments. Therefore, a three-year research was conducted in eastern Sicily aimed at studying the effects of four cover crop sequences (*Sinapis arvensis-Trigonella foenum-graecum-T. foenum-graecum*; *Medicago scutellata-Avena sativa-Lolium perenne*; *Vicia faba minor-A. sativa-A. sativa*; *A. sativa-V. faba. minor-L. perenne*) on weeds, major soil chemical properties and nutritional status of an organically grown orange orchard. The results highlighted that, among the studied cover crop sequences, *Vicia faba-Avena-Avena* was the most beneficial for weeds control within the orchard (92%, of cover crop cover, and 586 and 89 g DW $m^{-2}$ of cover crop aboveground biomass and weeds aboveground biomass, respectively). Overall, the chemical fertility of the soil was positively influenced. In particular, it was observed an increase of the content of total nitrogen and available phosphorus in the soil by both *Sinapis-Trigonella-Trigonella* (0.75 g $kg^{-1}$ and 59.0 mg $kg^{-1}$, respectively) and *Vicia faba-Avena-Avena* (0.70 g $kg^{-1}$ and 56.0 mg $kg^{-1}$, respectively) cover crop sequences. *Medicago-Avena-Lolium* sequence seemed to be the most useful to ensure a better nutritional status of the orange orchard.

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## Introduction

The growing concern about the negative side-effects of modern agriculture, has stimulated the need to find new agronomic solutions in order to improve the ecological profile of agrosystems (Carvalho Mendes and De Tourdonnet, 2013; Mauro *et al*., 2014). In this framework, cover crops can offer a valuable contribution for the agronomic management of modern agrosystems, since the positive effects that can generate on yield characteristics as well as lowering the environmental impact in farming systems (Lu *et al*., 2000; Djigal *et al*., 2012; Scopel *et al*., 2013). In this view, cover cropping has been proposed as a pivotal tool of improving agricultural sustainability, since in the soil it has the potential to mitigate compaction and erosion (Mitchell *et al*., 1999; Scopel *et al*., 2013), increase porosity (Carof *et al*., 2007), improve the ability to retain and remobilise nutrients (Doltra and Olesen, 2013), enrich organic matter content, especially in the case of legume species (Stagno *et al.,* 2008), enhance the macrofauna activity (Blanchart *et al*., 2006), release non-available phosphorus (Kamh *et al*., 1999), stimulate the generalist predator arthropod populations and heartworm communities. Moreover, cover crops generally reduce pest and weed pressure on cash crops (Den Hollander *et al*., 2007; Hiltbrunner *et al*., 2007; Pelosi *et al*., 2009; Chen *et al*., 2011; Campiglia *et al*., 2012) and consequently, can also represent a key option in the agronomic management of orchards in Mediterranean-type environments (Mauromicale *et al*., 2010; Mauro *et al*., 2011, 2014). However, these beneficial functions, particularly relevant under organic and low-input cropping regimes, require a knowledge about the adaptability of the species for the different agro-ecological conditions, with special attention about the changes that occur on native flora, soil properties and fruit trees (Mauro *et al*., 2013). For these reasons, a research was carried out in order to assess the effects of different cover crops sequences on weeds, major soil chemical characteristics and nutritional status of an organically grown orange orchard.

## Materials and methods

A three-year experiment, from 2011-12 to 2013-14, was conducted in eastern Sicily (37° 34′ N 14° 54′ E, 225 m asl) within a ~25 year-old orange orchard [*Citrus* x *sinensis* (L.) Osbeck, cultivar *Tarocco comune*] grafted on a *C.* x *aurantium* L. rootstock organically grown, at a planting density of 400 plant $ha^{-1}$ (5x5 m tree spacing). The climate of the area is Mediterranean semiarid, characterised by mild and wet winter, and warm and dry summer. The soil of the area is classified as *Vertic xerofluvents* according to soil taxonomy. At the start of the experiment, the soil was sampled and analysed for texture (39% clay, 28% silt, 33% sand), macronutrients content (0.55 g $kg^{-1}$ total N, 47.0 mg $kg^{-1}$ $P_2O_5$ and 461 mg $kg^{-1}$ exchangeable $K_2O$), organic matter (9.8 g $kg^{-1}$), total $CaCO_3$ (9.6%) and pH (7.2), according to the Italian official methods (Italian Regulation, 1999). Therefore, the soil was clay-loam and had low level of total N, organic matter and total Ca carbonate, and high phosphorus (P) and potassium (K) level, with carbon/nitrogen ratio (C/N) equal to 10.36.

Throughout the three years, the following four sequences of cover crop species were implemented within the orchard:

- ($S_1$) *Sinapis arvensis* L. (ocal landrace)-*Trigonella foenum-graecum* L. (local landrace)-*T. foenum-graecum* L. (local landrace);
- ($S_2$) *Medicago scutellata* L. (*Kelson*)-*Avena sativa* L. (local landrace)-*Lolium perenne* L. (*Popeye*);
- ($S_3$) *Vicia faba* L. subsp. *minor* Beck (*Prothabon*)-*A. sativa* L. (local landrace)-*A. sativa* L. (local landrace);
- ($S_4$) *A. sativa* L. (local landrace)-*V. faba* subsp. *minor* (*Prothabon*)-*L. perenne* L. (*Popeye*).

These treatments were attributed to experimental units of 100 m$^2$ three times replicated and arranged according to a randomised-block design.

In each year, cover crops were hand seeded in late October at rate of 45 seeds m$^{-2}$ for *V. faba minor* and 500 seeds m$^{-2}$ for the other species. In late spring, when the sward reached the maximum growth and cover crops species were at the full flowering stage, the plant coverage was estimated as a proportion of soil covered by the cover crop through three independent visual assessments. Afterward, eight sampling areas of 0.25 m$^2$ (0.5x0.5 m) randomly selected within each experimental unit were harvested cutting the sward at about 0.1 m above ground. For each collected sample, the cover crop species were separated from the weeds and the two components were weighed separately. These two subsamples were weighed again after drying in a thermo-ventilated oven at 105°C until constant weight, in order to determine their contribution in terms of dry biomass and the cover crop/weeds ratio was derived.

At the end of June 2014, ten soil samples per plot were taken by means of a 4 cm (i.d.) core auger to a depth of 0.3 m, in order to evaluate the overall effects of cover crop sequences on the main soil chemical properties. The soil samples were analysed according to the Italian official methods (Italian Regulation, 1999).

For the chemical analyses of tree leaves, a representative sample (6-7 month-old) were collected at the beginning of November from the end unfruiting branch of five trees (Embleton *et al*., 1973). A subsample (at least 15 leaves) was immediately freeze-dried (Christ freeze drier, Osterode am Harz, Germany), ground and used for the determination of total chlorophyll content (Uddling *et al*., 2007). The remaining leaves

**Table 1. Cover crops and weeds characteristics over the three-year experiment.**

| Years | Cover crop ground cover (%) | | | | Cover crop sequence<br>Cover crop above ground biomass (g DW m$^{-2}$) | | | | Weeds aboveground biomass (g DW m$^{-2}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ($S_1$) | ($S_2$) | ($S_3$) | ($S_4$) | ($S_1$) | ($S_2$) | ($S_3$) | ($S_4$) | ($S_1$) | ($S_2$) | ($S_3$) | ($S_4$) |
| 2011-2012 | *Sin* | *Med* | *Vic* | *Ave* | *Sin* | *Med* | *Vic* | *Ave* | *Sin* | *Med* | *Vic* | *Ave* |
| Means | 80$^b$ | 74$^c$ | 89$^a$ | 94$^a$ | 421$^c$ | 323$^d$ | 642$^b$ | 692$^a$ | 265$^a$ | 254$^a$ | 92$^b$ | 71$^b$ |
| 2012-2013 | *Tri* | *Ave* | *Ave* | *Vic* | *Tri* | *Ave* | *Ave* | *Vic* | *Tri* | *Ave* | *Ave* | *Vic* |
| Means | 43$^c$ | 94$^{ab}$ | 97$^a$ | 89$^b$ | 401$^c$ | 540$^b$ | 577$^b$ | 653$^a$ | 211$^a$ | 118$^b$ | 87$^c$ | 88$^c$ |
| 2013-2014 | *Tri* | *Lol* | *Ave* | *Lol* | *Tri* | *Lol* | *Ave* | *Lol* | *Tri* | *Lol* | *Ave* | *Lol* |
| Means | 36$^c$ | 28$^d$ | 91$^a$ | 50$^b$ | 327$^b$ | 115$^d$ | 538$^a$ | 254$^c$ | 218$^b$ | 202$^b$ | 88$^c$ | 308$^a$ |
| 3-year means | 53$^c$ | 65$^c$ | 92$^a$ | 77$^b$ | 383$^c$ | 326$^d$ | 586$^a$ | 533$^b$ | 231$^a$ | 191$^b$ | 89$^d$ | 156$^c$ |

*Sin, Sinapis arvensis* L.; *Med, Medicago scutellata* L.; *Vic, Vicia faba* L. subsp. *minor* Beck; *Ave, Avena sativa* L.; *Tri, Trigonella foenum-graecum* L.; Lol, *Lolium perenne* L. [a,b,c,d]Within each year and 3-year, means followed by different letters in the same row indicate significant differences at P<0.05 (*F*-protected least significant difference test).

**Table 2. Chemical characteristics of soil and orange tree leaves at the end of the experiment (June 2014) under the four cover crop sequences.**

| Chemical constituent | Cover crop sequence<br>($S_1$)<br>*Sin - Tri - Tri* | ($S_2$)<br>*Med - Ave - Lol* | ($S_3$)<br>*Vic - Ave - Ave* | ($S_4$)<br>*Ave - Vic - Lol* |
|---|---|---|---|---|
| Soil | | | | |
| Organic matter (g kg$^{-1}$) | 10.3$^b$ | 11.2$^{ab}$ | 12.9$^a$ | 11.2$^{ab}$ |
| Total N (g kg$^{-1}$) | 0.75$^a$ | 0.60$^b$ | 0.70$^{ab}$ | 0.60$^b$ |
| Assimilable P (mg kg$^{-1}$) | 59.0$^a$ | 42.5$^b$ | 56.0$^a$ | 46.0$^b$ |
| Orange tree leaves | | | | |
| Total chlorophyll (mg g$^{-1}$ FW) | 3.0$^b$ | 3.4$^a$ | 2.6$^c$ | 2.9$^{bc}$ |
| Ca (mg 100 g$^{-1}$ DW) | 408$^c$ | 658$^a$ | 486$^b$ | 503$^b$ |
| K (mg 100 g$^{-1}$ DW) | 302$^b$ | 311$^b$ | 335a | 326$^a$ |
| Mg (mg 100 g$^{-1}$ DW) | 88$^b$ | 114$^a$ | 95$^b$ | 83$^c$ |
| Na (mg 100 g$^{-1}$ DW) | 20$^b$ | 23$^a$ | 17$^c$ | 16$^c$ |
| Fe (mg 100 g$^{-1}$ DW) | 3.6$^a$ | 2.1$^c$ | 2.1$^c$ | 2.2$^c$ |
| Mn (mg 100 g$^{-1}$ DW) | 0.23$^a$ | 0.17$^c$ | 0.24$^a$ | 0.19$^b$ |
| Cu (mg 100 g$^{-1}$ DW) | 0.23$^a$ | 0.24$^a$ | 0.17$^a$ | 0.23$^a$ |

*Sin, Sinapis arvensis* L.; *Tri, Trigonella foenum-graecum* L.; *Med, Medicago scutellata* L.; *Vic, Vicia faba* L. subsp. *minor* Beck; *Ave, Avena sativa* L.; Lol, *Lolium perenne* L; N, nitrogen; P, phosphorus; Ca, calcium; K, potassium; Mg, magnesium; Na, sodium; Fe, iron; Mn, manganese; Cu, copper. [a,b,c]Values followed by different letters in each row indicate significant differences at P<0.05 (*F*-protected least significant difference test).

were oven-dried at 65°C (Binder, Milan, Italy) until a constant weight was reached. Then, the dehydrated material was ground and used for the determination of mineral profile according to the AOAC official method (1995). All the reagents and solvents, of analytical or high-performance liquid chromatography grade, were purchased from Sigma-Aldrich (Milan, Italy). All the chemical analyses were performed in triplicate.

Shapiro-Wilk's and Levene's tests were used to assess preliminarily the data for normal distribution and homoscedasticity, respectively, and the one-way ANOVA ($P \leq 0.05$) was applied. Percentage data were Bliss' transformed before the ANOVA (untransformed data are reported). Multiple mean comparisons were performed through Fisher's protected least significant difference test ($P \leq 0.05$).

## Results and discussion

Among the tested cover crop sequences, on average of the three years, $S_3$ (*Vicia faba-Avena-Avena*) highlighted the highest degree of ground cover as well as the greater amount of aboveground biomass, although the latter over the three years has not always been significantly higher, since in the first two years it has been higher for $S_4$ (*Avena-Vicia faba-Lolium*) (Table 1). By contrast, regardless the years, a significantly higher amount of weeds biomass were achieved for $S_1$ (*Sinapis-Trigonella-Trigonella*) followed by $S_2$ (*Medicago-Avena-Lolium*). Consequently, irrespective of the years, also the cover crops/weeds ratio, as a simple index of competitive ability of cover crops against weeds, was higher for $S_3$ and $S_4$ (6.6 and 6.0, respectively) compared to that found for S1 and S2 (1.7 and 2.1, respectively) (data not shown). Among the studied plant species, overall, the more competitive against weeds were oat within the *Poaceae* and field bean within the *Fabaceae* (Campiglia *et al*., 2012; Mauro *et al*., 2013). The same two plant species appear to have ensured a better degree of complementarity in $S_3$ cover crop sequence.

The analysis of the main chemical characteristics of the soil revealed overall an enhancement passing from the start to the end of the experiment, except that for assimilable phosphorus as a result of $S_2$ and $S_4$ treatments (Table 2). Nevertheless, the differences between the cover crop sequences were not always significant (Stagno *et al*., 2008). In particular, organic matter level was greater for $S_3$ compared to that of $S_1$, which anyway not differed from that observed for $S_2$ and $S_4$. However, the values of the latter two treatments were statistically similar to that of $S_3$ as well. The content of total nitrogen was significantly higher for $S_1$ and $S_3$, but the value observed for the latter treatment not differed statistically from those of the other ones. Assimilable P was found significantly higher for both $S_1$ and $S_3$ compared to the other cover crop sequences (Doltra and Olesen, 2013). The C/N ratio, which was equal to 10.36 at the start of the experiment decreased only for $S_1$ (7.50), in which two years of *Trigonella* followed one year of *Sinapis*, as compared to the other cover crop sequences (10.79, on average), which instead, led to a slight increase of this index (data not shown).

Leaf analysis, although limited to the end of the three-years of trials, allowed assessing the differences in the nutritional status of the orange trees in relation to the cover crop sequences. $S_2$ lead to the highest content of total chlorophyll in the leaves of trees. There are little knowledge concerning the variability of mineral composition of orange tree leaves in relation to different environmental and agronomic conditions, and specific information about *Tarocco comune* cultivar grown in Sicily under organic regime are unavailable. Therefore, for a comparison of our results of trees nutritional status we considered as source the reference values (deficient, low, optimal, high and excessive) of average concentrations of macro and micronutrients reported for the orange orchard by Embleton *et al*. (1973). According to these Authors, our data highlighted, overall, a critical nutritional status of the orchard, because the concentration of the minerals analysed was found below the threshold of deficiency. However, adopting $S_2$ cover crop sequence the highest mineral content of Ca, Mg and Na was reached, whereas $S_3$ and $S_4$ lead to a higher content of K in the leaves. $S_1$, instead, resulted in a higher content of Fe and Mn in the tree leaves, although the latter element was found similar to $S_3$. The four studied cover crop sequences similarly affected the Cu content of tree leaves.

Based on the findings obtained by this research, which require further confirmation, it can be argued that, in the organically grown orange orchard under the eastern Sicilian environmental conditions, among the different sequences of cover crop studied, *Vicia faba-Avena-Avena* was the most effective in terms of ground cover and weeds control. Overall, the chemical fertility of the soil was positively influenced, although not univocally related to the cover crop sequences, which with the exception of the *Sinapis-Trigonella-Trigonella*, have contributed to maintain an optimal C/N ratio. However, it is also worth mentioning the tendency toward an increase of the content of total nitrogen and available phosphorus in the soil by the two cover crop sequences *Sinapis-Trigonella-Trigonella* and *Vicia faba-Avena-Avena*. Moreover, the sequence *Medicago-Avena-Lolium*, despite the conditions of general nutritional deficiency observed in the orange orchard, seemed to be the most appropriate to ensure a better status of the trees.

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