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 Medicago-Avena-Lolium sequences (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.

Abstract

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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 (Cardol 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 (Kamb 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 (Mauronicale 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 crop 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 xerochrepts 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 P₂O₅ and 461 mg kg⁻¹ exchangeable K₂O), organic matter (9.8 g kg⁻¹), total CaCO₃ (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:
- (S1) Sinapis arvensis L. (local landrace)-Trigonella foenum-graecum L. (local landrace)-T. foenum-graecum L. (local landrace);
- (S2) Medicago scutellata L. (Kelson)-Avena sativa L. (local landrace)-Lolium perenne L. (Popeye);
- (S3) Vicia faba L. subsp. minor Beck (Prothaban)-A. sativa L. (local landrace)-A. sativa L. (local landrace);
- (S4) A. sativa L. (local landrace)-V. faba subsp. minor (Prothaban)-L. perenne L. (Popeye).

These treatments were attributed to experimental units of 100 m² three times replicated and arranged according to a randomized-block design.

In each year, cover crops were hand seeded in late October at rate of 45 seeds m⁻² for V. faba and 500 seeds m⁻² for the other species. In late spring, when the sward reached the maximum growth and cover crop 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 m² (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 | Weeds aboveground biomass (g DW m⁻²) |
|--------------|-----------------------------|---------------------|--------------------------------------|
|              | (S1) Sin, Med, Vic, Ave     | (S1) Sin, Med, Vic, Ave | (S1) Sin, Med, Vic, Ave               |
| 2011-2012    | 36c                          | 28d                 | 91a                                   |
| Means        | 43                           | 44                  | 44                                    |
| 2012-2013    | 38                           | 32                  | 64b                                   |
| Means        | 40                           | 54                  | 57b                                   |
| 2013-2014    | 39                           | 34                  | 64b                                   |
| Means        | 40                           | 53                  | 53b                                   |

### 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 | (S1) Sin - Tri - Tri | (S2) Med - Ave - Lol | (S3) Vic - Ave - Ave | (S4) Ave - Vic - Lol |
|----------------------|----------------------|----------------------|----------------------|----------------------|
| Organic matter (g kg⁻¹) | 10.3b              | 11.2ab              | 12.9a                | 11.3b                |
| Total N (g kg⁻¹)     | 0.75a               | 0.60b               | 0.70ab               | 0.60b                |
| Assimilable P (mg kg⁻¹) | 59.0b             | 42.5b               | 56.0b                | 46.0b                |
| Orange tree leaves   |                      |                      |                      |
| Total chlorophyll (mg g⁻¹ FW) | 3.0b              | 3.4a               | 2.6b                  | 2.9ac                |
| Ca (mg 100 g⁻¹ DW)    | 408b              | 635b                | 488b                 | 500b                 |
| K (mg 100 g⁻¹ DW)     | 302b              | 31.0b               | 335b                 | 328b                 |
| Mg (mg 100 g⁻¹ DW)    | 88b               | 114                  | 95b                  | 83c                  |
| Na (mg 100 g⁻¹ DW)    | 20b                | 23                    | 17c                   | 16c                  |
| Fe (mg 100 g⁻¹ DW)    | 3.6c              | 2.1c                  | 2.1c                | 2.1c                |
| Mn (mg 100 g⁻¹ DW)    | 0.23b             | 0.17b                 | 0.24b              | 0.19b               |
| Cu (mg 100 g⁻¹ DW)    | 0.23c             | 0.24c                 | 0.17c               | 0.23c               |

*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.*

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).
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<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<0.05).

Results and discussion

Among the tested cover crop sequences, on average of the three years, S1 (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 S1 (Avena-Vicia faba-Lolium) (Table 1). By contrast, regardless the years, a significantly higher amount of weeds biomass were achieved for S1 (Sinapis-Trigonella-Trigonella) followed by S2 (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 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 S1 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 S3 and S4 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 S3 compared to that of 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 S1 cover crop sequence.

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mass and the control of weeds. Eur. J. Agron. 26:21-9.

Italian Regulation, 1999. Ministero delle Politiche Agricole e Forestali - Decreto Ministeriale 13 settembre 1999 n. 185. Approvazione dei Metodi ufficiali di analisi chimica del suolo. In: G.U. n. 248, 21/10/1999.

Kamh M, Horst WJ, Amer F, Mostafa H, Maier P, 1999. Mobilization of soil and fertilizer phosphate by cover crops. Plant Soil 211:19-27.

Lu YC, Watkins KB, Teasdale JR, Abdul-Baki AA, 2000. Cover crops in sustainable food production. Food Rev. Int. 16:121-57.

Mauro RP, Pesce GR, Mauromicale G, 2013. The role of cover crops in agro-ecosystems management. In: A. Taab (ed.), Weeds and their ecological functions. Nova Science Publishers, Inc., Hauppauge, New York, NY, USA, pp 115-152.

Mauro RP, Occhipinti A, Longo AMG, Mauromicale G, 2011. Effects of shading on chlorophyll content, chlorophyll fluorescence and photosynthesis of subterranean clover. J. Agron. Crop Sci. 197:57-66.

Mauro RP, Sortino O, Dipasquale M, Mauromicale G, 2014. Phenological and growth response of legume cover crops to shading. J. Agric. Sci. 152:917-31.

Mauromicale G, Occhipinti A, Mauro R, 2010. Selection of shade-adapted subterranean clover species for cover cropping in orchards. Agron. Sustain. Develop. 30:473-80.

Mitchell JP, Thomsen CD, Graves WL, Shennan C, 1999. Cover crops for saline soils. J. Agron. Crop Sci. 183:167-78.

Pelosi C, Bertrand M, Roger-Estrade J, 2009. Earthworm community in conventional, organic and direct seeding with living mulch cropping systems. Agron. Sustain. Develop. 29:287-95.

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Stagno F, Abbate C, Intrigliolo F, Abbate V, Gennari M, 2008. Effect of leguminous cover crops on soil biological activity in pots of Citrus unshiu Marcovitch. Ital. J. Agron. 3:183-90.

Uddling J, Gelang-Alfredsson J, Piikki K, Pleijel H, 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth. Res. 91:37-46.