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Effectiveness of living mulch strategies for winter organic cauliflower (*Brassica oleracea* L. var. *botrytis*) production in Central and Southern Italy

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Abstract

In crop rotations, cover crops planted either before or concurrent with a main crop and maintained as a living ground cover (living mulch, LM) may provide many beneficial ecosystem services, and can be defined as 'agro-ecological service crops' (ASC). The objective of this research was to study the suitability of burr medic (*Medicago polymorpha* L. var. *anglona*) as an LM for organic cauliflower (*Brassica oleracea* L. var. *botrytis*) production in a Mediterranean environment. Two LM sowing times (early sowing – sowing concurrent with cauliflower transplanting versus late sowing – 3 weeks later) compared with a no living mulch control (LM-CT) were investigated in central Italy (Experiment 1), along with a comparison between two local cauliflower cultivars and a hybrid. In Southern Italy (Experiment 2), crop performance under two LM sowing times [20 days before cauliflower transplanting versus concurrent sowing (CS)] compared with a no LM-CT , with organic fertilizers and amendments allowed in organic farming as subplots, was assessed. In Experiment 1, no competition was observed between the late-sown ASC and the cash crop. An increase in crop nitrogen (N) uptake and weed mitigation was also determined in this treatment. There was a mixed response when comparing cultivar and LM interactions, with the hybrid cultivar in the late-sown LM producing the greatest yield. In Experiment 2, weather conditions had the greatest effect on crop response. However, an inverse trend between growth of the cash crop and the LM crop was observed in the CS treatment. A positive effect of LM introduction was found, particularly in altering the competitive relationship for N between the cash crop and weeds. In addition, yield results showed that, in LM systems, commercial organic fertilizers could be replaced with locally available organic fertilizers and amendments without any yield penalty. The effectiveness of LM strategies will thus depend on several factors: type of LM, cultivar of vegetable, weather, soils, length of growing season and ability to plant the cash crop into the LM. Initial research suggests the potential for burr medic as a LM for Mediterranean winter vegetable systems, but additional research is needed to ensure the viability of LM systems for longer periods of time.

Key words: Agroecological service crops, *Medicago polymorpha* L., cover crops, cauliflower cultivars, organic fertilizers

Introduction

Species diversity has been evaluated as a tool to improve agroecosystem resilience to perturbation, control weeds and pest occurrence, and preserve in-field biodiversity (Wezel et al., 2014). To implement the agroecological practices that are based on diversification, a redesign of existing cropping systems, which are relatively undiversified, would be required. This approach can be carried out by integrating suitable species in crop rotations, including intercrops. Intercropping is the coexistence of two or more crop species at the same time with different spatial arrangements in the same field (Malézieux et al., 2009). In particular, living mulches (LMs) are cover crops planted either before or with a main crop and maintained as a living ground cover throughout the growth cycle (Hartwig and Ammon, 2002). In sustainable agricultural systems, it is well documented that intercropping using LM can provide many beneficial ecosystem services (Campiglia et al., 2011); thus, they have recently been defined as 'agroecological service crops' (ASC) according to Canali et al. (2015). The ASC grown as LM are reported to immobilize excess soil nutrients, thus preventing nitrate leaching and improving soil physical characteristics (Carof et al., 2007); suppress weeds, reducing synthetic herbicide use (Teasdale et al., 2007; Tabaglio et al., 2008); promote biodiversity in the field (Fageria et al., 2005); add soil organic matter; and contribute to reducing surface water runoff, as well as loss of nutrients and pesticides (Hartwig and Ammon, 2002). Intercropping with ASC may be a valuable approach for weed management, particularly in organic farming, where weed control is one of the greatest concerns (Bilalis et al., 2010). Also, with a leguminous crop in the ASC, nitrogen (N) is fixed from the atmosphere and increases resource use efficiency in the cropping system (Wezel et al., 2014). Among leguminous ASC, self-reseeding annual legumes (such as *Medicago* spp.), which can persist over years without the need for reseeding, could play an important role in Mediterranean organic farming systems (Driouech et al., 2008). However, the most significant risk a farmer faces in using LM systems is the potentially negative interaction with the cash crop. According to Masiunas (1998), the success of such systems depends on the capacity to rapidly establish a ground cover that competes with weeds, without depleting the main resources of light, water and nutrients needed by the associated crop. Thus, many attempts to use LM in annual cropping systems have resulted in reduced yields of the cash crop (Kolota and Adamczewska-Sowińska, 2004; Hiltbrunner et al., 2007; Chase and Mbuya, 2008).

Compared with conventional systems, organic agroecosystems have additional features that require studying individual components and their interactions occurring across space and time (Bàrberi, 2002). The objective of this research was to study the suitability of burr medic (*Medicago polymorpha* L. var. *anglona*) as a LM in organic cauliflower (*Brassica oleracea* L. var. *botrytis*) systems at two sites in a Mediterranean environment. In Experiment 1, two local cauliflower cultivars and an F1 hybrid were compared within the organic intercropping system in Central Italy. Since vegetable crops have high nutrient demand and low efficiency of nutrient utilization, crop performance of LM systems for organic cauliflower combined with organic fertilization was assessed in Southern Italy in Experiment 2.

Materials and Methods

Study sites and experimental setup

The research was carried out during the 2010–2011 season at the following two sites: (1) Monsampolo del Tronto, in Central Italy (lat. 42°53′N; long. 13°48′E), at the MOnsampolo VEgetable organic long-term field experiment (MOVE LTE), which is located in the CRA-Research Unit for Vegetable Production (CRA-ORA); and (2) Metaponto (MT), in Southern Italy (lat. 40°24′N; long. 16°48′E), on the research farm 'Azienda Sperimentale Metaponto' of the CRA-Research Unit for Cropping Systems in Dry Environments (CRA-SCA, ASM).

Experiment 1. The Monsampolo site is characterized by a 'thermomediterranean' climate (Unesco-FAO, 1963). According to the soil taxonomy of the US Department of Agriculture (USDA, 1999), the soil type is a Typic Calcixerept fine-loamy, mixed thermic. The MOVE-LTE site was established in 2001 and is based on a 4-year crop rotation with different cover crops. More details about experiments at the MOVE-LTE site, system management, and agronomic and environmental performance are available in Campanelli and Canali (2012). The experimental design was a split-plot with two factors and three replications. Each main plot consisted of 16.8 m^2 of a LM of burr medic, *M. polymorpha* L. var. *anglona* at different sowing times: (i) concurrent sowing (CS; at cauliflower transplanting) and (ii) late sowing (LS; 3 weeks after cauliflower transplanting). These experimental treatments were compared with a no living mulch control (LM-CT). The LM-CT was weeded through two hoeings during the crop cycle (i.e., using standard organic farming agronomic practices in the area), while no weeding was performed in the CS and LS treatments past burr medic sowing. The burr medic was manually sown on August 28, 2010 (CS treatment) and on September 15, 2010 (LS treatment) at a rate of 80 kg ha^{-1}. Split-plots consisted of cauliflower cultivars (C): Emeraude F1 hybrid (EM), CRA-ORA1 and CRA-ORA2 (open-pollinated, locally adapted cultivars) and each treatment was repeated in triplicate. The cauliflower crop was manually transplanted on August 25, 2010 into rows with a 70 cm × 60 cm spacing. Harvests began on November 24 and terminated on December 28.

Experiment 2. The Metaponto site is characterized by an 'accentuated thermomediterranean' climate (Unesco-FAO, 1963). Soils are characterized as Typic Epiaquerts (USDA, 1999). The experiment was carried out according to a split–split-plot design where two factors were tested (Gomez and Gomez, 1984). The experimental area was divided into three blocks in a randomized complete block design, and planted to three (4 m × 20 m) vertical strips corresponding to LM (burr medic) sowing times: (i) early sowing (ES; 20 days before cauliflower transplanting) and (ii) CS (at cauliflower transplanting). These experimental treatments were compared with a no

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LM-CT. No weeding was performed in any of the treatments.

Each block was subsequently divided, in a randomized complete block design, into four horizontal (4 m × 5 m) strips corresponding to the following organic fertilizers and amendments (F), allowed in organic farming: (i) anaerobic digestate fertilizer, based on wine distillery wastewater (AD); (ii) composted municipal solid organic wastes from the city of Laterza ('Fertileva', Progeva Srl – Laterza, TA, Italy) (MSW); (iii) commercial humified organic fertilizer (ORG), based on dried cattle manure ('Italpollina' CRAI s.r.l., Rivoli Veronese, VR, Italy); compared with (iv) an unfertilized control (F-CT). Additional information about production processes for AD and MSW are reported in Montemurro et al. (2013). The organic materials (the same in both years) were applied to soil 1 month before cauliflower transplanting, at the rate of 100 kg N ha^{-1}. To account for the potential contribution of burr medic biological N fixation in the first two treatments, the fertilization applied in ES and CS was compared with an application rate of 200 kg N ha^{-1} of the same organic materials in the LM-CT. The cauliflower crop (cv. Triunphan) was manually transplanted on September 29, 2010 into the same in- and between-row spacing as Experiment 1 and was harvested on March 8, 2011.

Each burr medic sowing times × fertilizer combination plot (intersection plot) constituted a 20 m^2 area. Additional strips were included on the sides of the intersection plots in the experimental layout, to examine weed competition in detail, as follows: (i) weed stands without crop and ASC ('pure weed stands'; 1.5 m × 4.0 m) and (ii) crop stands without weeds and ASC ('pure crop stands' managed by manual weeding; 1.5 m × 2.0 m). Overall weed density was estimated in the weed seedling stage and weed species were identified over the cauliflower growing season.

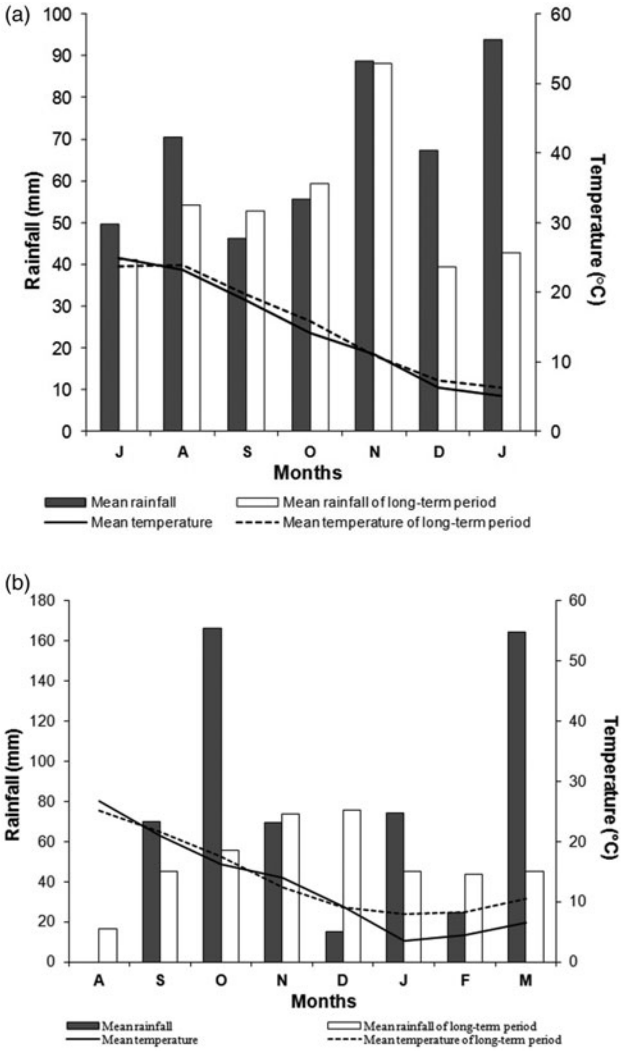

Figure 1. Weather conditions (total rainfall and monthly values of mean temperatures) recorded during the cauliflower growing season at Experiment 1, Monsampolo site (a), and at Experiment 2, Metaponto site (b). The values are compared with the rainfall and mean temperatures over a 30-yr period.

Measurements and statistical analysis

At harvesting, cauliflower heads were collected from three randomly selected plants in each plot and from the pure crop stands (Experiment 2) during the cash crop harvest period. Aboveground biomass of cauliflower, weeds and burr medic were separately measured in all plots and in pure stands. Cauliflower heads and entire plant residues including stalks cut at ground level, weeds and burr medic were dried at 70°C for 48 h to obtain dry weights. Cauliflower head and residue biomass were summed to obtain the total aboveground crop biomass. Nitrogen content (Kjeldahl, 1883) of cauliflower heads, crop residues and weeds was determined, based on this calculation of N uptake: plant N content × plant dry biomass (Ciaccia et al., 2015). The N content × burr medic biomass (burr medic N supply) was also calculated in order to measure the contribution of the ASC treatment to the N pool of the entire cropping system.

In order to measure the effect of the LM on competition for N between the cash crop and weeds, in Experiment 2, ΔN uptake (%), obtained from weed–crop treatments, using averages across repetitions, was used. The equations were:

$$\begin{aligned}&\text{total crop }\Delta\text{N uptake}\\&\quad = 100 - [(\text{N uptake of crop in presence of weeds}\\&\qquad \times 100/\text{N uptake of crop in pure stands})]\end{aligned} \tag{1}$$

$$\begin{aligned}\text{weed }\Delta\text{N uptake} = 100 - ((&\text{N uptake of weeds in}\\&\text{presence of crop } \times 100)/\\&\text{N uptake of weeds in pure stands})\end{aligned} \tag{2}$$

Soil mineral N (N-mineral = NO_3^-–N + NH_4^+–N, recorded at 0–30–cm soil depth) was determined at both experimental sites and in both years at the following sampling

Table 1. Effects of living mulch sowing times and cauliflower cultivars on cauliflower yield, cauliflower crop residue, weeds and burr medic biomass (t ha^{-1}) and effects on N uptake (kg ha^{-1}) in Experiment 1.

	Biomass (t ha^{-1})				N uptake (kg ha^{-1})			
	Cauliflower head yield	**Cauliflower crop residue**	**Weed**	**Burr medic**	**Cauliflower head**	**Cauliflower crop residue**	**Weed**	**Burr medic**
Living mulch (LM)								
LM-CT	1.10 b	7.85 a	0.26 ab	–	65.9 b	288.7 a	5.86 b	–
CS	1.34 b	4.89 b	0.36 a	1.56 a	78.5 b	184.9 b	10.30 a	41.5 a
LS	1.83 a	8.68 a	0.13 b	0.61 b	101.3 a	347.7 a	3.74 b	18.1 b
	***	***	**	***	**	*	**	***
Cultivar (C)								
EM	1.83 a	6.57	0.07 b	0.26 b	85.3	209.9 b	1.27 c	6.71 b
CRA–ORA1	1.16 b	7.74	0.29 a	1.68 a	73.1	306.1 a	6.75 b	32.9 a
CRA–ORA2	1.26 b	7.11	0.39 a	1.32 a	87.2	305.3 a	11.88 a	49.8 a
	**	n.s.	***	***	n.s.	***	***	**
Mean	1.42	7.14	0.25	1.09	81.9	273.8	6.64	29.8
LM × C	*	n.s.	***	**	***	n.s.	***	n.s.

Note: The probability levels are presented by living mulch sowing time, cultivar and their interactions. *, **, ***Significant at $P < 0.05$, 0.01 and 0.001, respectively. n.s. = nonsignificant (LM-CT = control, no living mulch; CS = concurrent sowing, at cauliflower transplanting; LS = late sowing, three weeks after cauliflower transplanting. EM = Emeraude F1 Hybrid; CRA-ORA1 and CRA-ORA2 = open-pollinated, locally adapted cultivars).

times: (I) cauliflower transplanting; (II) 5-leaf rosette stage; (III) head emergence; and (IV) at the end of the harvest period. Soil mineral N was extracted by 2 M KCl (1:10, w/v) and measured by continual flow colorimetry according to Krom (1980) and Henriksen and Selmer-Olsen (1970) for NH_4^+–N and NO_3^-–N, respectively.

Analysis of variance (ANOVA) was carried out in both experiments, with LM management and cultivar/fertilizer as factors. To compare differences obtained, means were further analyzed by Tukey's HSD test ($P < 0.05$). The selected analysis was performed by using SPSS 17.0 (SPSS Inc. Released 2008).

Results and Discussion

Weather conditions

At the Experiment 1 site, over the period of July–January 2010–2011, total rainfall (472 mm) was higher than the 30-yr long-term average (377 mm) (Fig. 1a). Mean temperatures were lower, at 14.7°C, than the long-term average of 15.4°C. At the Experiment 2 site, over the period of September–March 2010–2011, total rainfall (583 mm) was considerably higher than the long-term average (384 mm) (Fig. 1b). Conversely, the mean temperature was lower (10.7°C) than the 30-yr average of 12.4°C.

Effects of LM and crop cultivars on crop performance in central Italy

In Experiment 1, analysis of variance revealed overall significant main effects of LM sowing times and cultivar selection (C) on cauliflower head yield (Table 1). Late sowing increased head yield compared with CS and LM-CT. While sowing times also affected crop residue biomass production, cultivar effect on crop residue biomass was not significant. In particular, planting the LM concurrent with cauliflower transplanting, lowered cauliflower biomass by 43.6 and 37.7%, compared with sowing the LM 3 weeks after tranplanting, and the no LM-CT, respectively. Main effects of N uptake in cauliflower heads and crop residues were also found at the different LM sowing times, while C effects on cauliflower head N content were significant for crop residue, but not head content. At the same time, CS had the lowest N uptake in cauliflower residues. Regarding the response of cultivars, EM had the lowest residue N uptake, which was lower by 31.3% than the average of CRA-ORA1 and CRA-ORA2. Significant interactions were observed between LM and C regarding effects on cauliflower yield and yield N uptake (Fig. 2a and b), while cauliflower crop residue biomass and N uptake comparisons were not significant (Table 1). The combination of EM with LS, and of CRA-ORA1 with LM-CT, resulted in the highest and lowest yield, respectively (Fig. 2a). No significant differences were observed among the other LM × C treatments.

The most productive results were obtained when EM was grown in combination with the LS LM, which confirms the role of the LM sowing period as highlighted by other authors with different vegetable crops. Kolota and Adamczewska-Sowińska (2004) found that LM did not adversely affect plant growth and yield of leeks if LM planting was delayed until 11 weeks after transplanting, with a marketable yield reduction of 31% when LM sowing was only delayed 3–5 weeks after cash crop transplanting. Successful intercropping necessitates the

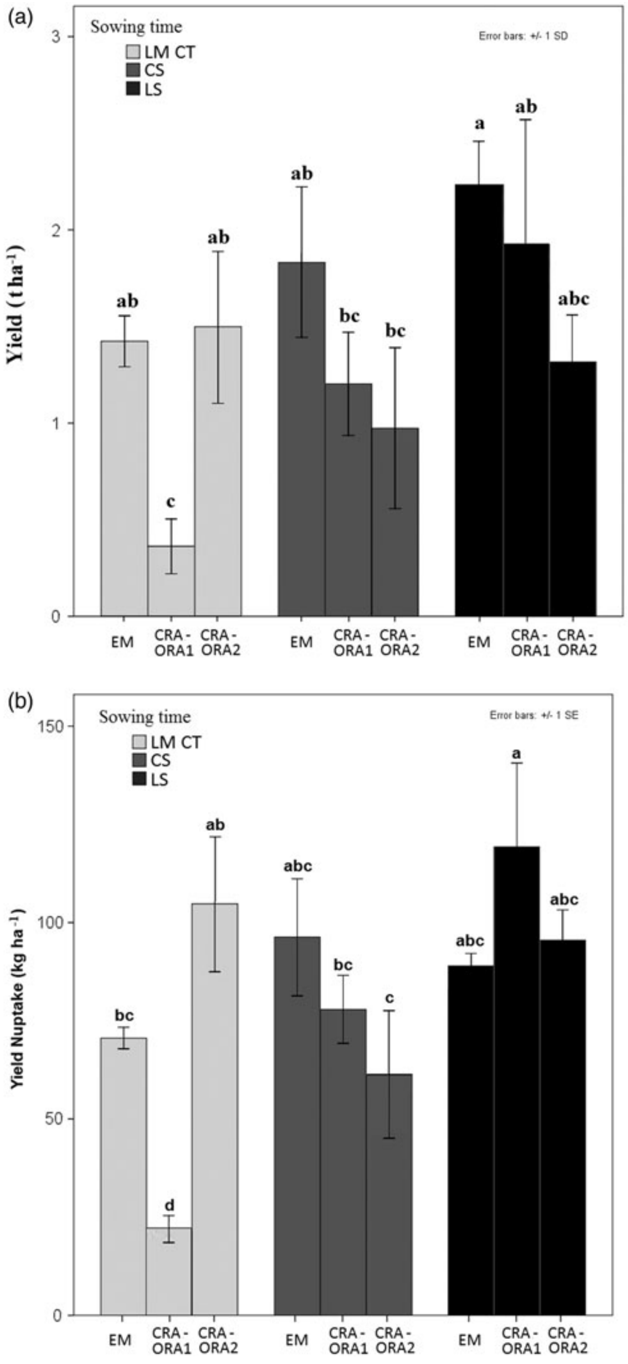

Figure 2. Interactions between LM sowing times and cauliflower cultivars on: (a) total yield biomass (t ha^{-1}) and (b) N uptake in cauliflower heads (kg ha^{-1}), at the Monsampolo site (Experiment 1). Bars with different letters are significantly different according to DMRT at the $P \leq 0.05$ probability level (LM-CT = control, no living mulch; CS, concurrent sowing, at cauliflower transplanting; LS, late sowing, 3 weeks after cauliflower transplanting. EM, Emeraude F1 Hybrid; CRA–ORA1 and CRA–ORA2, open-pollinated, locally adapted cultivars).

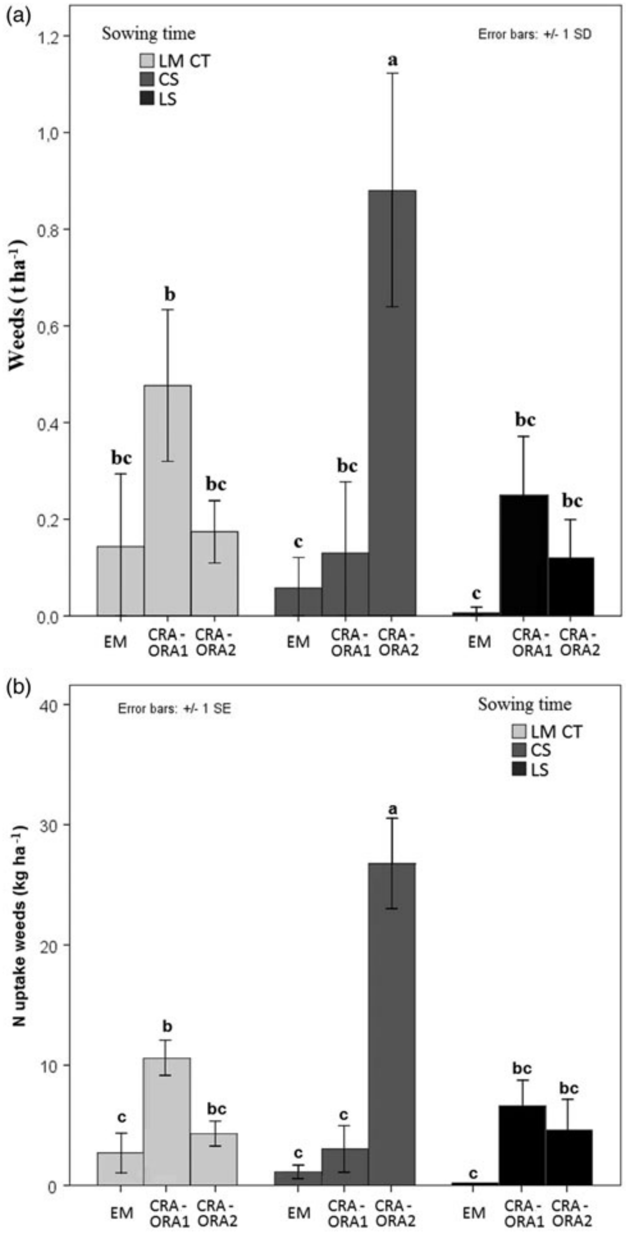

Figure 3. Interactions between LM sowing times and cauliflower cultivars on: (a) weed dry biomass (t ha^{-1}) and (b) N uptake in weeds (kg ha^{-1}), at the Monsampolo site (Experiment 1). Bars with different letters are significantly different according to DMRT at the $P \leq 0.05$ probability level (LM-CT, control, no living mulch; CS, concurrent sowing, at cauliflower transplanting; LS, late sowing, 3 weeks after cauliflower transplanting. EM, Emeraude F1 Hybrid; CRA–ORA1 and CRA–ORA2, open-pollinated, locally adapted cultivars).

efficient use of resources by interacting crops, which is often best achieved through temporal separation based on critical periods of nutrient demand (Santos et al., 2002), as shown by the highest crop N uptake in the LS treatment (Table 1). In particular, the CRA–ORA1–LS combination showed the greatest N uptake in cauliflower heads, which was about 430 and 53% higher than CRA–ORA1 with LM-CT and CS, respectively (Fig. 2b). In addition, the N uptake in cauliflower heads in

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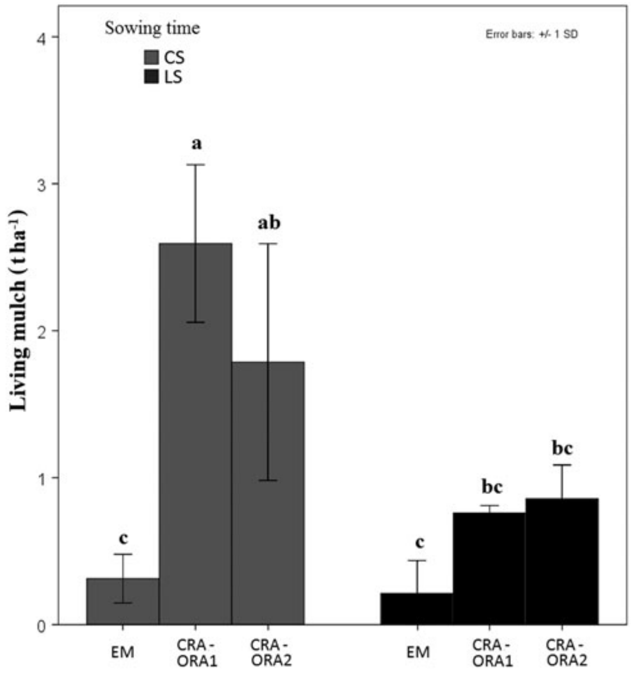

Figure 4. Interactions between LM sowing times and cauliflower cultivar on burr medic dry biomass (t ha^{-1}) at the Monsampolo site (Experiment 1). Bars with different letters are significantly different according to DMRT at the $P \leq 0.05$ probability level (LM-CT, control, no living mulch; CS, concurrent sowing, at cauliflower transplanting; LS, late sowing, 3 weeks after cauliflower transplanting. EM, Emeraude F1 Hybrid; CRA–ORA1 and CRA–ORA2, open-pollinated, locally adapted cultivars).

CRA–ORA1–LS plots was higher than the EM–LM-CT, and CRA–ORA2–CS plots by 68.9 and 94.4%, respectively.

The ANOVA revealed that weed biomass in the different LM treatments only differed between CS and LS, whereas significant main effects of LM sowing times and C on burr medic biomass were observed (Table 1). Main effects of N uptake in weeds and burr medic were also found. At this experiment site, CS resulted in the highest burr medic N uptake, which was 129% higher than the LS treatment. This parameter was significantly lower for the hybrid cultivar than for CRA–ORA2 (by 86.5%), and CRA–ORA1 (by 79.6%). This last result would suggest that EM, with a larger biomass, may have had a smothering effect on burr medic, further confirming the role of cultivars in LM competitiveness (Mohler, 2007).

In addition, two-way interactions (LM × C) were significant for N uptake in cauliflower heads and in weeds (Fig. 3a and b). There were no significant interactions between LM and C with burr medic N uptake. The EM–CS and EM–LS combinations had significantly lower weed biomass than the CRA–ORA2–CS plots (0.06 and 0.01 versus 0.88 t ha^{-1}, respectively). Similarly, the highest weed N uptake was found in the CRA–ORA2–CS plots, which was notably higher than EM under all LM treatments. Comparable and similar values were found for the other treatment combinations. Results determined that there was a trend toward greater weed biomass in CS plots, where the highest weed N uptake also was observed. This result suggests that, as a consequence of CS, the ASC was less effective in mitigating weed establishment and growth compared with LS, in particular in combination with CRA–ORA2.

The greatest burr medic biomass was found for both local cultivars under CS management, at 2.60 and 1.80 t ha^{-1} for CRA–ORA1 and CRA–ORA2, respectively, compared with the EM in combination with LS (0.21 t ha^{-1}) and CS (0.31 t ha^{-1}) treatments (Fig. 4). In agreement with other studies (den Hollander et al., 2007; Hiltbrunner et al., 2007), an inverse trend between the growth of the cash crop and that of the LM was detected. The highest burr medic biomass and N content (Table 1) was observed where the LM was concurrently sown in the local cultivar plots, which also had the lowest cauliflower biomass. Conversely, no significant differences were detected for LS burr medic biomass among cultivar plots. This result confirms that there was limited competition between the cash crop and the ASC in the LS treatment. Because of the faster growth pattern of the hybrid cultivar, greater competition with weeds and the LM was observed under both sowing times. These results support the idea of cultivar differences in crop competitiveness (Zimdahl, 2007) and in the effectiveness of LMs on potential suppression of weeds (Walters, 2011; Kolota and Adamczewska-Sowińska, 2013).

Effects of LM and organic fertilizers on crop performance in southern Italy

At this study site, there were two extreme rainfall events during the cauliflower cropping cycle (Fig. 1), which greatly influenced cultivation and reduced yields. Nevertheless, significant main effects of LM sowing time and fertilizer (F) on cauliflower head yield and residue biomass were found in Experiment 2 (Table 2). Among LM sowing times, cauliflower yields in the ES and CS treatments demonstrated greater production than in the LM-CT plots, by an average of 210%. There was a trend toward higher cauliflower crop residue (by 162%) in the CS treatment compared with the LM-CT treatment. Similarly, N uptake in cauliflower heads and residue in ES and CS treatments was higher than in the LM-CT treatment. An inverse trend between the growth of the cash crop and that of the ASC, highlighted by Hiltbrunner et al. (2007), was also observed in CS plots, showing that the time of LM sowing should be chosen properly to ensure optimal soil cover and intercropping efficiency (Müller-Schärrer and Potter, 1991).

Regarding fertilizer effects, the ORG treatment yields were 87% higher than the average production in the F-CT and AD fertilizer treatments, although yields were

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Table 2. Effects of living mulch sowing times and organic fertilization on cauliflower yield, cauliflower crop residue, weeds and burr medic biomass (t ha^{-1}) and effects on N uptake (kg ha^{-1}) in Experiment 2.

	Biomass (t ha^{-1})				N uptake (kg ha^{-1})			
	Cauliflower head yield	**Cauliflower crop residue**	**Weed**	**Burr medic**	**Cauliflower head**	**Cauliflower crop residue**	**Weed**	**Burr medic**
Living mulch (LM)								
ES	0.44 a	0.33 ab	0.92 b	2.03 a	11.10 a	8.74 a	28.16 b	71.45 a
CS	0.56 a	0.42 a	0.48 b	1.27 b	11.84 a	9.68 a	13.21 b	37.16 b
LM-CT	0.16 b	0.16 b	2.61 a	–	4.16 b	3.38 b	64.21 a	–
	***	*	***	**	*	**	***	**
Fertilizer (F)								
AD	0.33 b	0.27 ab	1.60	1.50	12.21	8.35	42.30	58.30
MSW	0.38 ab	0.31 ab	1.40	1.65	8.20	7.08	37.71	48.98
ORG	0.57 a	0.39 a	1.45	1.72	6.88	6.20	25.46	54.03
F-CT	0.28 b	0.23 b	0.91	1.72	8.85	7.43	35.31	55.92
	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean	0.39	0.30	1.34	1.65	9.03	7.27	35.20	54.31
LM × F	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Note: The probability levels are presented by living mulch sowing time, cultivar and their interactions. *, **, ***Significant at $P < 0.05$, 0.01 and 0.001, respectively. n.s. = nonsignificant (ES = early sowing, 20 days before cauliflower transplanting; CS = concurrent sowing, at cauliflower transplanting; LM-CT = no living mulch control; AD = anaerobic digestate fertilizer; MSW = composted municipal solid organic wastes; ORG = commercial humified fertilizer; F-CT = unfertilized control).

not significantly different from the MSW treatment. At the same time, the ORG treatment produced significantly higher cauliflower crop residues (0.39 t ha^{-1}) than the F-CT treatment (0.23 t ha^{-1}). The comparable yield results of MSW with ORG fertilizer indicated that both types of fertilization are viable options for organic farmers if available at the local level. Similarly, different studies at the same site have found that commercial organic fertilizers could be effectively replaced by locally available organic fertilizers and amendments (Montemurro et al., 2005, 2015).

Significant main effects of LM sowing times on weed and burr medic biomass and N uptake were found, but no effect of F was observed (Table 2). There were also no significant LM × F interaction effects (Table 2). The LM-CT treatment led to a significantly higher amount of weed biomass than the ES (by 184%) and CS (by 440%) treatments, which were equivalent. Similarly, the highest weed N uptake was found in the LM-CT treatment. The ES treatment showed significantly higher burr medic biomass and N uptake than the CS treatment (by 60 and 92%, respectively). The result observed for weed biomass and weed N uptake confirms the findings of Jędrszczyk et al. (2005), where the authors showed that cultivation of head cabbage with a white clover LM was associated with an extensive weed biomass reduction of 96%. However, no significant weed biomass reduction was recorded in the ES treatment compared with CS, despite the higher burr medic biomass observed in ES plots. This result is not in agreement with findings of Bàrberi et al. (2008) who showed a proportional decrease of weed biomass in relation to the amount of LM biomass in spinach cultivation.

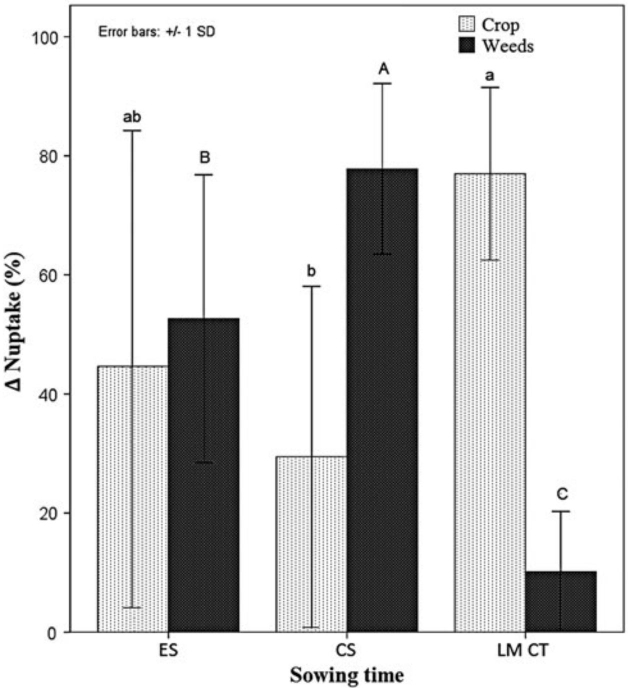

Figure 5. Effects of LM sowing times on ΔN uptake (%) of crop and weeds at the Metaponto site (Experiment 2). Bars with different letters are significantly different according to DMRT at the $P \leq 0.05$ probability level (LM-CT, control, no living mulch; ES, early sowing, 20 days before cauliflower transplanting; CS, concurrent sowing, at cauliflower transplanting).

Main effects of LM on ΔN uptake of crop and weeds were found (Fig. 5), while no effect of F and no LM × F interactions were found (data not shown). This last

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Table 3. Effects of living mulch sowing times and cauliflower cultivars on soil N-mineral at different sampling times (I–IV), at the Monsampolo site (Experiment 1).

	N-min (mg kg^{-1})			
	I	**II**	**III**	**IV**
Living mulch (LM)	n.s.	***	n.s.	*
Cultivar (C)	***	n.s.	n.s.	***
LM × C	*	**	n.s.	***
Interactions				
LM-CT–EM	58.01 a	47.14 bc	28.61	18.16 de
CS–EM	44.90 ab	79.94 a	34.81	21.99 cd
LS–EM	32.76 bc	38.74 c	31.63	25.21 c
LM-CT–CRA–ORA1	27.86 c	31.38 c	30.99	20.70 ce
CS–CRA–ORA1	35.64 bc	64.57 ab	31.65	16.28 f
LS–CRA–ORA1	34.80 bc	45.37 c	29.86	19.31 df
LM-CT–CRA–ORA2	37.01 bc	41.75 c	31.00	36.07 a
CS–CRA–ORA2	57.11 a	64.31 ab	28.61	30.79 b
LS–CRA–ORA2	58.92 a	66.30 a	32.00	32.11 b
Mean	43.00	53.28	31.02	24.51

Note: Mean values in each column followed by a different letter are significantly different according to Tukey's test.
n.s., non-significant (LM-CT, control, no living mulch; CS, concurrent sowing, at cauliflower transplanting; LS, late sowing, 3 weeks after cauliflower transplanting. EM, Emeraude F1 Hybrid; CRA-ORA1 and CRA-ORA2, open-pollinated, locally adapted cultivars). Sampling times: (I) cauliflower transplanting; (II) 5-leaf rosette stage; (III) head emergence and (IV) at the end of the harvest period.
*, **, ***, Significant at $P < 0.05$, 0.01 and 0.001, respectively.

Table 4. Effects of living mulch sowing times and fertilizers on soil N-mineral at different sampling times (I–IV), at the Metaponto site (Experiment 2).

	N-min (mg kg^{-1})			
	I	**II**	**III**	**IV**
Living mulch (LM)				
ES	47.90 a	36.50	29.07 b	38.45
CS	45.57 a	34.49	34.19 a	38.59
LM-CT	36.79 b	33.83	30.02 b	43.87
	*	n.s.	**	n.s.
Fertilizer (F)				
AD	47.22 ab	33.21	32.02	43.26
MSW	37.71 b	33.35	29.39	38.57
ORG	48.39 a	38.44	32.80	39.93
F-CT	40.35 ab	34.47	30.15	39.44
	*	n.s.	n.s.	n.s.
Mean	34.42	34.93	31.09	40.30
LM × F	n.s.	n.s.	n.s.	n.s.

Note: Mean values in each column followed by a different letter are significantly different according to Tukey's test.
n.s., non-significant (ES, anticipated sowing, 20 days before cauliflower transplanting; CS, concurrent sowing, at cauliflower transplanting; LM-CT, no living mulch control; AD, anaerobic digestate fertilizer; MSW, composted municipal solid organic wastes; ORG, commercial humified fertilizer; F-CT, unfertilized control). Sampling times: (I) cauliflower transplanting; (II) 5-leaf rosette stage; (III) head emergence and (IV) at the end of the harvest period.
*, **, ***, Significant at $P < 0.05$, 0.01 and 0.001, respectively.

result is in contrast with the findings of other authors who showed an improvement in weed control as a consequence of crop fertilization (Evans et al., 2003; Brainard and Bellinder, 2004). A higher value was observed for cauliflower ΔN uptake in LM-CT plots in comparison with the CS treatment. Conversely, CS plots showed the highest weed ΔN uptake and LM-CT the lowest. Regarding competition for N, both the ΔN uptake for cauliflower crop and weeds showed differences in N content between crops and weeds as a function of the LM sowing time. The CS plots had the lowest crop N uptake reduction and the highest for weeds, confirming the effect of delaying LM sowing on improving the ability of the crop to compete with weeds without interfering with crop yield (Brainard et al., 2004). The results suggest that, in certain LM systems, crop N loss could be reduced compared with systems in which weeding is performed. Contrastingly, weed N uptake in LM plots decreased significantly in comparison with pure crop stands. As a consequence of this response, weeding may be reduced to conserve N in the cropping system, which represents a benefit of adopting the LM technique.

Soil fertility

Regarding the influence of treatments on soils of the study sites, there were significant main effects of LM sowing times at sampling times II and IV on soil N-mineral in Experiment 1, and at sampling times I and IV for cultivar effects (Table 3). Overall, significant LM × C interactions were also found, except for the III sampling time. There was a trend toward higher soil N-mineral in the LM-CT–EM, CS–CRA–ORA2 and LS–CRA–ORA2 combinations at sampling time I, although these combinations were not significantly different from CS–EM (Table 3). No significant difference was observed among any other treatment combinations. At sampling II, the highest soil N values were obtained in CS–EM, LS–CRA–ORA2, as well as in CS–CRA–ORA1 and CS–CRA–ORA2, but the CS–CRA–ORA1 and CS–CRA–ORA2 combinations were not significantly different from the EM in LM-CT plots. No significant differences were found at sampling time III. Finally, at sampling time IV, the highest value was found for LM-CT–CRA–ORA2, while CS–CRA–ORA2 and LS–CRA–ORA2 had high soil N-mineral compared with the other treatment combinations. The combination of CRA–ORA1 with CS was associated with the lowest soil N value. As indicated by

Whitmore and Schröder (2007), N uptake in intercrops could be such that there would be less N available for leaching compared with sole crops at a similar total density. The observed amount of soil N-mineral could subsequently increase the risk of N loss by leaching, but this was not measured. This finding may be counter to other studies that highlighted that a leguminous LM may immobilize excess nutrients, thus preventing groundwater contamination (Hartwig and Ammon, 2002).

At the Experiment 2 site, analysis of variance revealed significant main effects of LM on soil N-mineral for sampling times I and III, and at sampling time I for fertilizer effects (Table 4). At sampling time I, LM-CT plots had the lowest soil N-mineral content, which was 21.3% lower than the average of CS and ES. Among fertilizer treatments, ORG plots had an N-mineral value (48.39 $\mathrm{mg\,kg^{-1}}$) that was significantly higher (28.3%) than MSW plots, but similar to the other treatments. At sampling time III, the CS treatment had the highest value, which was 13.9 and 17.6% higher than LM-CT and ES, respectively. The lack of significant differences among LM treatments (as well as among fertilizer treatments) for soil N-mineral remaining after harvest may have resulted from the unfavorable weather conditions, allowing N runoff and/or leaching.

Conclusions

Identifying the conditions that could lead to positive ecosystem services from LMs, such as weed suppression while minimizing crop competition, is of major interest to farmers and researchers. The results obtained in our study demonstrated that vegetable cropping systems designed in accordance with agroecological principles, by combining different agronomic strategies, including proper selection of the sowing time of the ASC, appropriate cash crop cultivars, and effective organic fertilizers and amendments, are able to sustain crop yields while mitigating weed pressure. In particular, results suggested that in central Italy, the LM was non-competitive with the cash crop, and demonstrated the potential to mitigate weed infestations, with the LS of the ASC. Moreover, different cultivars showed varying weed mitigation and competitiveness for resource use, depending on the LM sowing time. At the Experiment 2 site, an inverse trend between the growth of the cash crop and that of the ASC was observed for the CS treatment. However, weather conditions had the greatest effect on the intercropping system interactions at this study site. The differences between the two experiments highlighted the need to evaluate LM treatments in different environments. This research provided the framework for future investigations to be undertaken in different environments, aiming to evaluate the feasibility of introducing other LMs (e.g., mixing different types of ASC) and their effectiveness. The use of LM could help farmers maintain production and food security in spite of changes in temperature and rainfall, since cover crops can protect the soil from the impact of rain drops and modify soil temperatures. Therefore, further research on the use of LM as a potential technique in climate change adaptation should be encouraged, particularly for vegetable winter crop production under Mediterranean conditions.

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References

Bàrberi, P. 2002. Weed management in organic agriculture: Are we addressing the right issues? Weed Research 42:177–193.

Bàrberi, P., Bigongiali, F., Antichi, D., Carlesi, S., Fontanelli, M., Fiasconi, C., and Lulli, L. 2008. Innovative crop and weed management strategies for organic spinach: crop yield and weed suppression. In: Cultivating the future based on science. Vol. 1: Organic crop production. Proceedings of the Second Scientific Conference of the International Society of Organic Agriculture Research (ISOFAR). Modena, Italy, 18–20 June, 252–255.

Bilalis, D., Papastylianou, P., Konstantas, A., Patsiali, S., Karkanis, A., and Efthimiadou, A. 2010. Weed-suppressive effects of maize-legume intercropping in organic farming. International Journal of Pest Management 56:173–181.

Brainard, D.C., and Bellinder, R.R. 2004. Weed suppression in broccoli-winter rye intercropping systems. Weed Science 52: 281–290.

Brainard, D.C., Bellinder, R.R., and Miller, A.J. 2004. Cultivation and interseeding for weed control in transplanted cabbage. Weed Technology 18:704–710.

Campanelli, G. and Canali, S. 2012. Crop production and environmental effects in conventional and organic vegetable farming systems: The case of a long-term experiment in Mediterranean conditions (Central Italy). Journal of Sustainable Agriculture 36:599–619.

Campiglia, E., Mancinelli, R., and Radicetti, E. 2011. Influence of no-tillage and organic mulching on tomato (*Solanum lycopersicum* L.) production and nitrogen use in the Mediterranean environment of central Italy. Scientia Horticulturae 130:588–598.

Canali, S., Diacono, M., Campanelli, G., and Montemurro, F. 2015. Organic no-till with roller crimpers: Agro-ecosystem services and applications in organic Mediterranean vegetable productions. Sustainable Agriculture Research 4(3):66–75. doi: 10.5539/

Carof, M., de Tourdonnet, S., Saulas, P., Le Floch, D., and Roger-Estrade, J. 2007. Undersowing wheat with different living mulches in a no-till system. I. Yield analysis. Agronomy for Sustainable Development 27:347–356.

Chase, C.A. and Mbuya, O.S. 2008. Greater interference from living mulches than weeds in organic broccoli production. Weed Technology 22(2):280–285.

Ciaccia, C., Montemurro, F., Campanelli, G., Diacono, M., Fiore, A., Canali, S. 2015. Legume cover crop management and organic amendments application: Effects on organic zucchini performance and weed competition. Scientia Horticulturae 185:48–58.

den Hollander, N.G., Bastiaans, L., and Kropff, M.J. 2007. Clover as a cover crop for weed suppression in an intercropping design II. Competitive ability of several clover species. European Journal of Agronomy 26:104–112.

Driouech, N., Abou Fayad, F., Ghanem, A., and Al-Bitar, L. 2008. Agronomic performance of annual self-reseeding legumes and their self-establishment potential in the Apulia region of Italy. 16th IFOAM Organic World Congress, Modena, Italy, June 16–20, 2008. Available at Web site http://orgprints.org/view/projects/conference.html

Evans, S.P., Knevic, S.Z., Lindquist, J.L., Shapiro, C.A., and Blankenship, E.E. 2003. Nitrogen application influences the critical period for weed control in corn. Weed Science 51: 408–417.

Fageria, N.K., Baligar, V.C., and Bailey, B.A. 2005. Role of cover crops in improving soil and row crop productivity. Communications in Soil Science and Plant Analysis 36: 2733–2757.

Gomez, K.A. and Gomez, A. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley and Sons, New York, NY.

Hartwig, N.L. and Ammon, H.U. 2002. Cover crops and living mulches. Weed Science 50:688–699.

Henriksen, A. and Selmer-Olsen, A.R. 1970. Automatic methods for determining nitrate and nitrite in water and soil extracts. Analyst 95:514–518.

Hiltbrunner, J., Liedgens, M., Bloch, L., Stamp, P., and Streit, B. 2007. Legume cover crops as living mulches for winter wheat: Components of biomass and the control of weeds. European Journal of Agronomy 26:21–29.

Jędrszczyk, E., Poniedziałek, M., and Sękara, A. 2005. Effect of living mulch on white head cabbage (*Brassica oleracea* var. *capitata* subvar. *alba* L.) yielding. Folia Horticulturae 17 (2):29–36.

Kjeldahl, J. 1883. Neue Methods zur Bestimmung des Stickstoffs in Organischen Korpern, Z. Analytical Chemistry 22: 366–382.

Kolota, E. and Adamczewska-Sowińska, K. 2004. The effects of living mulches on yield, overwintering and biological value of leek. Acta Horticulturae 638:209–214.

Kolota, E. and Adamczewska-Sowińska, K. 2013. Living mulches in vegetable crops production: Perspectives and limitations (A reviev). Acta Scientiarum Polonorum Hortorum Cultus 12(6):127–142.

Krom, M.D. 1980. Spectrophotometric determination of ammonia: A study of a modified Berthelot reaction using salicylate and dichloroisocyanurate. Analyst 105:305–316.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier- Lafontaine, H., Rapidel, B., de Tourdonnet, S., and Valantin-Morison, M. 2009. Mixing plant species in cropping systems: Concepts, tools and models. A review. Agronomy for Sustainable Development 29:43–62.

Masiunas, J.B. 1998. Production of vegetables using cover crop and living mulches: A review. Journal of Vegetable Crop Production 4:11–31.

Mohler, C.L. 2007. Enhancing the competitive ability of crops. In M. Liebman, C.L. Mohler and C.P. Staver (eds). Ecological Management of Agricultural Weeds. Cambridge University Press, Cambridge, UK. p. 269–321.

Montemurro, F., Convertini, G., Ferri, D., and Maiorana, M. 2005. MSW compost application on tomato crops in Mediterranean conditions: Effects on agronomic performance and nitrogen utilization. Compost Science & Utilization 13(4):234–242.

Montemurro, F., Fiore, A., Campanelli, G., Tittarelli, F., Ledda, L., and Canali, S. 2013. Organic fertilization, green manure, and vetch mulch to improve organic zucchini yield and quality. Hortscience 48:1027–1033.

Montemurro, F., Tittarelli, F., Lopedota, O., Verrastro, V., and Diacono, M. 2015. Agronomic performance of experimental fertilizers on spinach (*Spinacia oleracea* L.) in organic farming. Nutrient Cycling in Agroecosystems 102:227–241.

Müller-Schärrer, H. and Potter, C.A. 1991. Cover plants in field grown vegetables: prospects and limitations. Brighton Crop Protection Conference-Weeds, 599–604.

Santos, R.H.S., Gliessman, S.R., and Cecon, P.R. 2002. Crop interactions in broccoli intercropping. Biological Agriculture and Horticulture 20:51–75.

Soil Survey Staff. 1999. Soil Taxonomy. Agriculture Handbook 436, USDA–NRCS, Washington, DC.

SPSS Inc. 2008. SPSS Statistics for Windows, Version 17.0. SPSS Inc., Chicago, IL.

Tabaglio, V., Gavazzi, C., Schulz, M., and Marocco, A. 2008. Alternative weed control using the allelopathic effect of natural benzoxazinoids from rye mulch. Agronomy for Sustainable Development 28:397–401.

Teasdale, J.R., Brandsæter, L.O., Calegari, A., and Skora Neto, F. 2007. Cover crops and weed management. In M.K. Upadhyaya and R.E. Blackshaw (eds). Non-Chemical Weed Management. CAB International Wallingford. p. 49–64.

Unesco-FAO. 1963. Etude Écologique de la Zone Méditerranéenne. Carte Bioclimatique de la zone Méditerranéenne [Ecological study of the Mediterranean area: Bioclimatic map of the Mediterranean sea]. Paris-Rome, 60.

Walters, S.A. 2011. Weed management systems for no-tillage vegetable production. In S. Soloneski and M.L. Larramendy (eds). Herbicides, Theory and Applications. InTech, Rijeka, Croatia. pp. 17–40.

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

Whitmore, A.P. and Schröder, J.J. 2007. Intercropping reduces nitrate leaching from under field crops without loss of yield: A modelling study. European Journal of Agronomy 27:81–88.

Zimdahl, R. (ed.) 2007. Weed management using the principles of competition. In Weed-Crop Competition: A Review. 2nd ed. Blackwell Publishing, Hoboken. pp. 146–166.