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Effects of olive shoot residues on shoot and root growth of potted olive plantlets

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ABSTRACT

Decomposition of olive shoot residue (OSR) from leaf shedding and pruning may provide nutrient to olive orchards, although beyond a phytotoxic threshold it can also hamper plant growth. We studied OSR decomposition effects on plant growth, biomass partitioning and soil fertility. Four levels of OSR (0%, 3%, 10% and 30% [v/v]) were mixed into the substrate and placed close to the roots compared on two olive potted cultivars over 240 days using a destructive sampling approach. Organic matter, polyphenol and nitrogen contents in the substrate, fine root respiration and electrolyte leakage, leaf pigment content, chlorophyll *a* fluorescence, biomass partitioning, fine root nutritional status were determined. OSR increased the content of organic matter, polyphenols and nitrogen in the soil. In the first 150 days, OSR beyond 3% induced autotoxic effects, and altered fine root respiration, and electrolyte leakage and biomass allocation. After 240 days, OSR induced a stimulatory effect on fine roots and shoot growth and increased shoot and fine root nitrogen content. Application OSR did not significantly altered leaf pigment content and chlorophyll *a* fluorescence. As a conclusion, above the threshold of 3%, olive cannot prevent autotoxicity during the early decomposition of OSR, but later soil fertility and plant growth can be increased.

1. Introduction

Addition of litter to the soil can influence both the nutrients and the phytotoxins. Litter decomposition governs the nutrient levels and the carbon cycle, and consequently influences the physico-chemical properties of the soil, which are the key components to maintain the productivity of natural and agro-ecosystems. In the natural ecosystem, the greater portion of the net primary production is shed as plant litter and enters the decomposition pathway. Humification to a stable organic form and mineralization of plant litter into its elemental components is thus responsible for the replenishment of the pool of plant-available nutrients, thereby maintaining the productivity of these ecosystems (Swift et al., 1979; Zucconi, 2003).

However, when the accumulation of residues from a single crop is overcoming a physiological threshold, it disrupts the humification process, inducing odd decompositions that delay stabilization and release toxic metabolites (Zucconi et al., 1984). These in turn can induce specific dispathic (detrimental or negative) effects that account for 'soil sickness' (Zucconi and De Bertoldi, 1987; Zucconi, 1993). The sensitivity of roots to the phytotoxic substances means that in addition to root competition (external function), phytotoxins might be also reduces root functioning, spacing of roots belonging to the same plant (internal function) (Falik et al., 2005). Root absorption, in particular, can be hindered by these phytotoxins (Zucconi et al., 1984; Zucconi, 2003), which promote dystrophies, root die-back, and eventually disease and abnormal root transmigration and ramification in the crop, or crops, being cultivated (Neri et al., 1996, 2005; Bonanomi et al., 2006; Giorgi et al., 2008; Polverigiani et al., 2014).

Litter decomposition rate and its role in nutrient dynamics according to environmental conditions, litter chemical characteristics and biotic factors, have all been widely studied (Coûteaux et al., 1995; Hättenschwiler and Gasser, 2005). Within any ecosystem, the plant litter quality and diversity are the main factors that affect the rate of litter decomposition (Aerts, 1997; Cadisch and Giller, 1997; Harguindeguy et al., 2008; Bonanomi and Incerti, 2010; Gangatharan and Neri, 2012). However, in tropical climates, the major regulator of litter decomposition is the rainfall pattern (Anderson and Swift, 1983), whereas temperature is the most limiting factor in temperate climates. Most frequently, in tropical ecosystems, the dry season is the time when the plants shed their

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Table 1
Changes in total organic matter, organic carbon, total N content, C/N ratio and polyphenols of growth substrate during the study period.

Uprooting time (days)	Olive shoot residue (%)	Total organic matter (g/kg)	Organic carbon (g/kg)	Total nitrogen (g/kg)	C/N ratio	Polyphenols (μg/kg)
0	Control	197.5	114.6	4.4	26.1	858.7*
	3	237.0	137.5	4.5	30.6	
	10	281.7	163.4	4.5	36.4	
	30	319.9	185.6	5.7	32.0	
	**SE	±20.7	±15.4	±0.3	±2.1	
30	Control	259.3	150.4	3.9	38.6	52.2
	3	296.2	171.8	5.0	34.4	86.3
	10	305.4	177.2	5.8	30.6	89.5
	30	315.8	173.3	7.2	24.1	110.8
	**SE	±12.3	±6.0	±0.7	±3.1	±12.1
150	Control	236.4	136.3	3.4	40.1	45.3
	3	237.0	137.5	4.2	32.8	57.3
	10	240.3	139.4	4.5	31.0	59.5
	30	320.9	186.1	7.4	25.2	88.9
	**SE	±20.8	±12.1	±0.8	±3.0	±9.2
240	Control	246.8	143.2	3.6	39.8	39.6
	3	260.0	150.8	4.8	31.4	46.8
	10	283.1	164.2	5.3	3.0	49.3
	30	286.6	168.6	6.8	24.8	61.6
	**SE	±9.4	±5.9	±0.7	±1.8	±4.6

* Polyphenols content of OSR.
** SE: standard error of the four treatments.

leaves. However, the dry hot conditions inhibit leaf decomposition, and a large amount of dead leaf litter accumulates on the forest floor, with decomposition starting with the onset of rain.

In the Mediterranean agro-ecological zone, olive-leaf shedding and shoot pruning usually happen at the end of the cold winter season, the decomposition starts with the onset of spring, and litter layers in natural plant communities are generally made up of more than one species. Most olive agro-ecosystems are devoted to mono-cropping, with little or no chance of crop rotation because of the nature of the crop, such as olive orchards that remain for decades or for centuries in the same field. The actual changes in the system of cultivation are from traditional (100–200 trees per hectare) to intensive (400–500 trees per hectare), and to super-high density (≥1000 trees per hectare) olive orchards (Pastor et al., 2007). The incorporation of pruned shoots (leaves and branches) into the soil as organic amendments is wide spreading to reduce the external fertilization supply. Thus farming intensification, coupled with the incorporation of increasing amounts of OSR from pruning, may create a new burden on the root system by inducing phytotoxic responses (autopathy) and nitrogen (N) immobilization during litter decomposition (Zucconi et al., 1984; Pastor et al., 2007; Bonanomi et al., 2011).

The objective of the present study was to investigate the autotoxic effects of OSR on shoot and root growth of potted olive plantlets during litter decomposition, and to determine whether there is any direct relationship between root stress induced by OSR decomposition and several physiological parameters associated with plant growth.

2. Materials and methods

2.1. Plant material and growth substrate

The experiment was carried out from August 2010 until April 2011 in a controlled greenhouse of the Experimental farm of Polytechnic University of Marche. One-year-old plantlets of olive (*Olea europaea* L.) cvs. Arbequina (the most used Spanish cultivar in super-high density orchards) and Frantoio (one of the most widespread Italian cultivar) were grown in 3.0-l plastic pots (0.14 × 0.14 × 0.18 m) filled with growth substrate (20% soil, 30% blend of wheat and frozen through black sphagnum peat, 50% blond sphagnum peat), plus ground and sieved OSR. The sieved OSR were incorporated into the growth substrate at 0% (control), 3%, 10% and 30% (v/v) to provide four treatment levels. Care was taken to ensure the contact of the roots with the OSR. The chemical characteristics of the growth substrate before the start of the experiment were reported in Table 1 at time zero.

The OSR were collected from a mature olive tree orchard after pruning in December 2009, and dried in the sun for 21 days and roughly ground into smaller pieces, which were further dried in the sun for 15 days. To stimulate rapid decomposition and release of nutrients and phytotoxins, these sun-dried OSR were ground to dust and sieved (to 3 mm). The sieved OSR were stored in a dry place before the beginning of the experiment, to reduce microbial degradation (Fig. 1a and b).

Before transplanting, the roots were washed under gently flowing tap water in a laboratory sink, to avoid any effects of the primary growth substrate (Fig. 1c and d).

2.2. Experimental design and green house conditions

A total of 72 olive plantlets (36 of each of the two varieties) were used, in a randomized block design with three replicates per treatment, for three uprooting dates (30, 150, 240 days after planting). For each uprooting date, one plant from each block per treatment and cultivar (for a total of 12 plants of Arbequina and 12 plants of Frantoio) was taken for the destructive measurements.

The temperature and relative humidity of the greenhouse were recorded on an hourly basis, using 175-H2 Testo sensors and a data logger (Testo, Germany). The climatic trend in the greenhouse throughout the whole experiment period is reported in Fig. 2.

2.3. Above-ground and below-ground measurements

On each uprooting date, the basal stem diameter and plant height were measured using digital calipers and a ruler, respectively. The fresh and dry weights (with drying in oven at 70 °C for 48 h) of the above-ground (stem, branches, leaves) and below-ground biomass were determined for each plant. The below-ground biomass was further divided into three groups according to the root diameter: fine roots (≤2 mm), small roots (2 to 5 mm), and coarse roots (≥5 mm). The uproots of each plant were placed on a stack of sieves of decreasing diameter (3.0–0.25 mm) and washed thoroughly in a laboratory sink with gently flowing tap water. Then the

Fig. 1. Preparation of fresh OSR. Sun dried and partially ground (a), ground and sieved with 3-mm sieve (b), washed olive plantlet before replanting to reduce effects of original substrate (c), and transplanted to a new pot (d).

coarse, small and fine roots were picked out and placed separately in de-ionized water, based on their diameters.

All of the washed roots were then oven dried at 70 °C to constant weight, and the dry mass was determined for each root class. The means of the fine root dry mass and the total root dry mass per treatment were calculated. The shoot and fine root relative growth rate were determined as the slopes of the natural logarithms of the shoot dry mass and the fine root dry mass *versus* time, as mg g^{-1} dry weight d^{-1} (Hunt, 1982). The data were then expressed as inhibition of shoot and root growth (*i.e.*, percentage differences in shoot and root mass, and height and diameter) compared with the control.

2.4. Root respiration and electrolyte leakage

The fine root respiration was expressed as O_2 consumption at 20 °C, and this was measured with Clark-type oxygen electrodes (Hansatech Oxygraph, Kings Lynn, UK) connected to constant temperature circulating water. For each treatment at each uprooting date, the fine roots of the first group that were free from soil were rinsed and collected, and then immediately immersed in 5 mM of 2-(*N*-morpholino) ethalesulfonic acid buffer solution (MES) of pH 5.5.

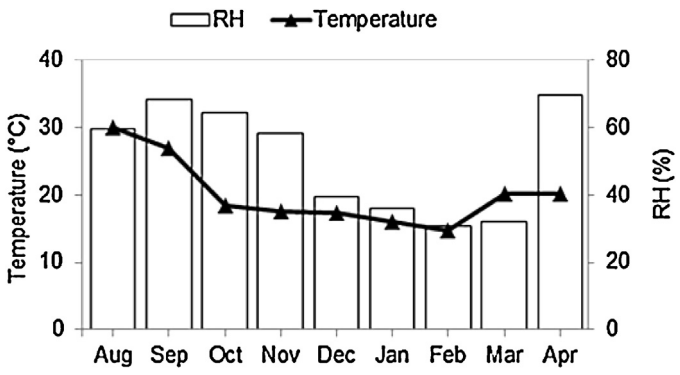

Fig. 2. Relative humidity (RH) and average temperatures recorded in the greenhouse during the study period.

Two root samples per plant were inserted in an Oxygraph chamber and the oxygen depletion inside the chamber was recorded until the steady slope that displayed the O_2 consumption rate was identified (about 10 min). Then roots were removed from the chamber, dried for 48 h at 70 °C, and then weighed.

Root electrolytic leakage can be used as an indicator of stress (Huang et al., 2005) and cell membrane stability and integrity (Martin et al., 1987). The fine root electrolyte leakage was determined using the relative conductivity method (Wilner, 1955, 1960), as modified by Mckay (1992). The fine roots from each treatment at each uprooting period were washed in cold tap water to remove the soil, and rinsed in de-ionized water to remove surface ions. Then these roots were immersed in de-ionized water of known electrical conductivity (ECde-ionized water) and the EC of the water was measured at time zero (EC0, immediately after immersion of the roots), after 30 min of immersion (EC30), and after boiling the sample in the de-ionized water for 5 min and cooling to the same temperature and volume (ECtotal). The fine root electrolyte leakage was calculated using the following formula: fine root electrolyte leakage (%) = 100 × (EC30 − ECinitial)/(ECtotal − ECinitial), where ECinitial = EC0 − ECde-ionized water.

2.5. Chlorophyll content and chlorophyll a fluorescence

Chlorophyll *a* fluorescence reflects the leaf photosynthesis intensity as it arises from absorbed light energy that is not used for photosynthetic reactions and heat dissipation (Oxborough, 2004). The relative chlorophyll concentrations were estimated using a SPAD-502 chlorophyll meter (Minolta, Osaka, Japan). The meter takes instant readings without destroying the plant tissue, and the readings are performed in a very short time. This optical device determines the greenness and the interaction of thylakoid chlorophyll with incident light (Jifon et al., 2005). The chlorophyll meter readings (SPAD values) were repeatedly taken for two mature leaves of each plantlet at the three apices of a triangle inscribed in the leaf lamina, for every uprooting period.

Leaf chlorophyll *a* fluorescence was quantified on leaves using a Fim-1500 chlorophyll fluorometer (Analytical Developmental Company Ltd., England). The measuring system applies an array

of red-light-emitting diodes of peak wavelength 470 nm for saturating light pulses that provide even illumination over the exposed area of the leaf (4 mm diameter). During application of a saturation pulse, the initial fluorescence yield (F_o) and the maximum fluorescence yield (F_m) were assessed in dark adapted leaves over 20 min. The maximum efficiency of the PSII represented the number of electrons transported by a PSII reaction center per mole of quanta absorbed by PSII. The PSII maximum efficiency was calculated according to Maxwell and Johnson (2000) and Jifon and Syvertsen (2003), as: PSII = F_v/F_m for dark adapted leaves. For each leaf, the values of the selected fluorescence parameters are expressed as means. The chlorophyll fluorescence measurements were carried out on two mature leaves of each plantlet before uprooting.

2.6. Analysis of growth substrate, leaves and fine roots

Samples of the growth substrate were taken from each treatment at each uprooting period, and stored in a deep-freeze (−18 °C) until used for analysis. The total organic matter (TOM) was determined by combustion in a furnace at 550 °C for 4 h. The total organic carbon (TOC) was determined by dry combustion (TOC Analyser multi N/C 1000). The total N (TN) was determined following the Kjeldahl N determination method (Tan, 1996). Hence, plant phenolics are one of major groups of phytotoxins released during decomposition of organic residues (Einhelling, 1995; Jose, 2002), the total polyphenols of the growth substrate and OSR were quantified using Folin–Ciocalteu reagent at each uprooting period.

To determine the nutritional status of the olive plantlets, the fine-roots and leaf samples were washed with an aqueous solution of citric acid (2%) and oven dried at 70 °C for 12 h. The N content was determined by the Kjeldhal method, after acid digestion (nitric acid: perchloric acid, 2:1 [v/v]). Phosphorus (P) was measured after acid digestion and color-metering of the P as molybdovanadate phosphoric acid (Murphy and Riley, 1962).

2.7. Data analysis

To study the interaction effects of the OSR treatments, the olive cultivars and duration of OSR decomposition (uprooting day), the data were subjected to two-way ANOVA. To determine whether the application of different OSR treatments on the shoot and root growth and the physiology of the olive plantlets were significantly different, the data were then analyzed according to the Duncan multiple range test, using the STATISTICA software package (Statsoft, Tulsa, OK).

3. Results

3.1. Shoot and root growth

Incorporation of OSR close to the roots had significant inhibiting effects on the shoot growth of *O. europaea* L. plantlets (Table 2) at the first uprooting date (30 days after planting), showing higher negative effects with higher OSR levels. At the second date (150 days) the negative effects were increased with 10 and 30% OSR while they disappeared with 3% OSR. After 240 days the negative effects disappeared in all the treatments (Fig. 3a). Accordingly, the 3% OSR treatment induced inhibitory effects on the shoot dry mass and basal stem diameter expansion, of 8.6% and 1.3%, respectively, at the first uprooting date, and they induced stimulatory effects that increased with time and reached 12.5% for the shoot dry mass and 4.7% for the basal stem diameter expansion, as compared to the control (Fig. 3a and b). No inhibitory effects were seen on plant height throughout the study period for 3% OSR, although a stimulatory effect was seen after 150 days that reached a peak of 3.6%, as compared to the control (Fig. 3c). For the 10% and 30% OSR treatments, the inhibitory effect of OSR decomposition was greater at the initial uprooting date, and increased up to 150 days, reaching 15.7% and 26.9%, respectively, for the shoot dry mass (Fig. 3a), 7.7% and 16.2%, respectively, for the basal stem diameter (Fig. 3b), and 4.5% and 12.5%, respectively, for the plant height (Fig. 3c), as compared to the control.

The addition of OSR temporarily inhibited also root growth with a similar trend (Fig. 3d and e). For the 3% OSR treatment, the inhibitory effects on fine root dry mass and total root dry mass (the sum of the fine, small and coarse roots) were minimal (8.0% and 3.8%, respectively, as compared to the control), and this was detected after the first 30 days. Later, 3% OSR induced stimulatory effects irrespective of root type. For the 10% OSR treatment, the inhibitory effects on fine root and total root dry mass were intermediate and reached their maximum levels after 30 days; stimulatory effects were then induced on the fine root growth, although the inhibition of total root growth remained. The inhibitory effects of the 30% OSR treatment were greater and increased to 150 days, reaching 34.5% and 31.9% for the fine root and total root dry mass, respectively, as compared to the control (Fig. 3d and e). This 30% OSR also induced transmigrating root growth and lenticels development of the coarse and small roots, and for the basal portion of the stem (data not shown). The negative effects on root growth disappeared after 240 days.

Table 2
Summary of the two way ANOVA (univariate test of significance) for the main and interactive effects of the OSR treatments on fine root mass, total root mass, and shoot dry weight.

Factor	Variable				
	df	SS	MS	*F*	*p*
Fine-root mass					
Uprooting time (T)	2	237.27	118.64	184.77	<0.001
Cultivar (C)	1	1.15	1.15	1.79	0.187
Olive shoot residue (OSR)	3	18.46	6.15	9.58	<0.001
T × C	2	0.05	0.03	0.04	0.961
T × OSR	6	12.52	2.09	3.25	0.009
C × OSR	3	2.42	0.80	1.25	0.300
T × C × OSR	6	6.03	1.01	1.57	0.177
Total-root mass					
Uprooting time (T)	2	969.65	484.82	121.24	<0.001
Cultivar (C)	1	13.50	13.50	3.38	0.072
Olive shoot residue (OSR)	3	76.66	25.55	6.39	<0.001
T × C	2	1.05	0.53	0.13	0.877
T × OSR	6	41.66	6.94	1.74	0.133
C × OSR	3	11.88	3.96	0.99	0.405
T × C × OSR	6	11.60	1.93	0.48	0.817
Shoot dry weight					
Uprooting time (T)	2	1745.26	872.63	145.97	<0.001
Cultivar (C)	1	512.27	512.27	85.69	<0.001
Olive shoot residue (OSR)	3	181.80	60.60	10.14	<0.001
T × C	2	9.43	4.72	0.79	0.460
T × OSR	6	245.05	40.84	6.83	<0.001
C × OSR	3	18.12	6.04	1.01	0.396
T × C × OSR	6	23.36	3.89	0.65	0.689

In each test, all of the experimental factors were considered as categorical predictors, whereas the uprooting times were considered as independent continuous variables. df: degrees of freedom, SS: sum of squares, MS: mean square, *F*: *F*-test, *p:* probability.

3.2. Shoot-to-root ratio, and shoot and fine root relative growth rates

For each uprooting date, for all of the treatments, the ratios between the shoot dry mass and total root dry mass were not significantly different. The shoot-to-root ratio decreased after 240 days and reached similar values in all the treatments (Fig. 4).

The application of OSR changed the trends of both the shoot and fine root relative growth rate during the study period (Fig. 5a and b). The shoot relative growth rate was highly impaired and reached 0.5

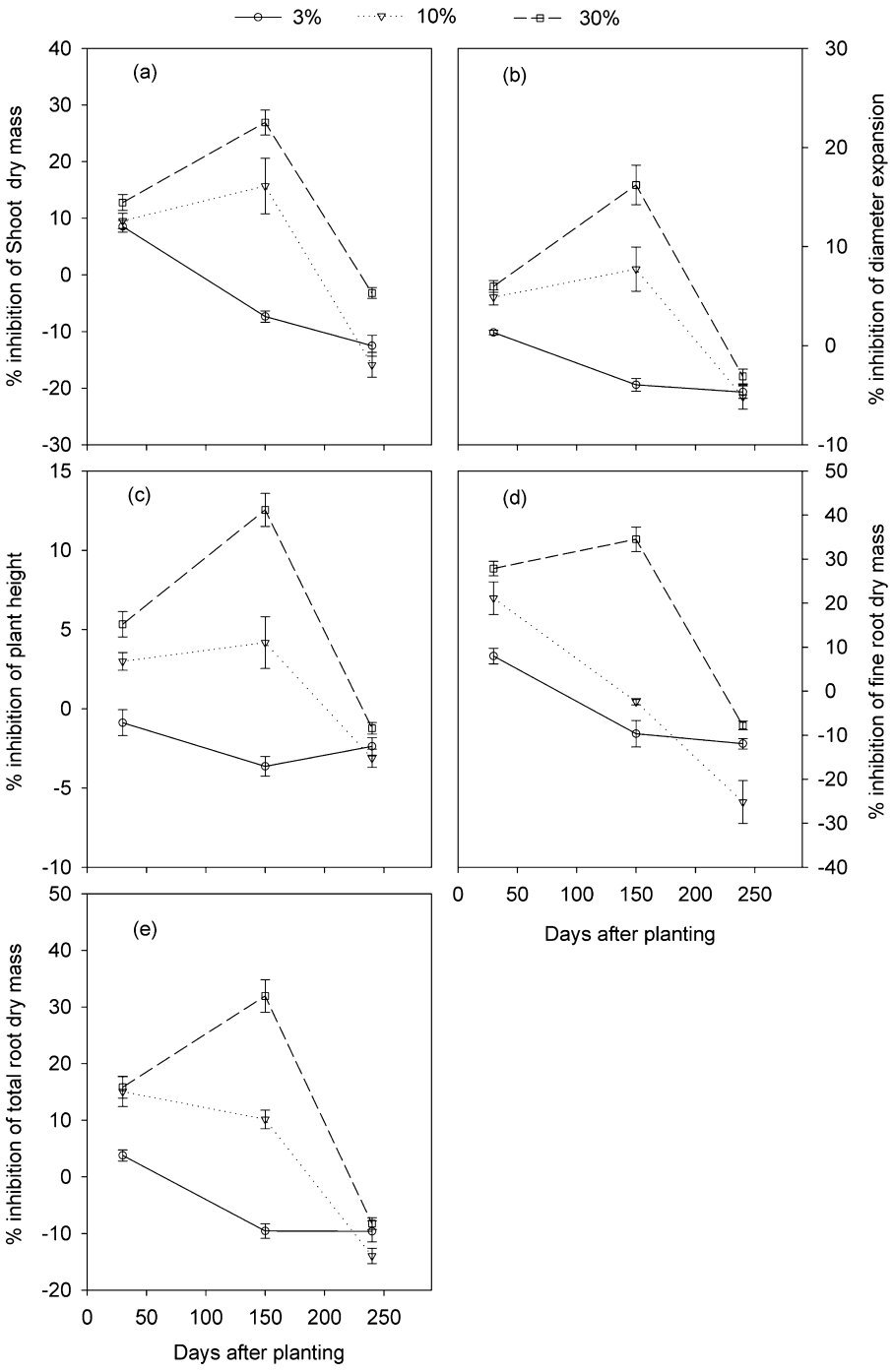

Fig. 3. Inhibitory effects of different OSR treatments on shoot dry mass (a), height (b), diameter (c), fine root dry mass (d), and total root dry mass (e), as compared to the control (100%), at different stage of decomposition. Data are means ± SE ($n = 6$). Values above and below zero indicate inhibitory and stimulatory effects, respectively.

and $-0.8\,mg\,g^{-1}\,d^{-1}$ for the 10% and 30% OSR treatments, respectively, in the first 30 days, and then increased up to the following uprooting date, reaching a maximum ($4.6\,mg\,g^{-1}\,d^{-1}$) by the end of the experimental period. In contrast, the control and the 3% OSR treatments reached their maximum relative growth rates after 30 and 150 days, respectively (Fig. 5a).

As compared to the shoot relative growth rate, the fine root relative growth rate was greatly inhibited by the OSR decomposition in the first 30 days. In particular, for the 10% and 30% OSR treatments, the fine root relative growth rate was reduced by 2.3 and $6.2\,mg\,g^{-1}\,d^{-1}$ in the first 30 days, and then increased for the following sampling dates. For the 3% OSR treatment, the fine root relative growth rate showed a similar trend to that of the control (Fig. 5b).

3.3. Root leakage and respiration

The fine root electrolyte leakage for the 3%, 10% and 30% OSR treatments increased to 150 days from the planting date, and then decreased to the end of the experimental period, with higher rates for the first two uprooting dates, as compared to the control: for the 30% OSR treatment by 29.1%, followed by 19.6% for the 10% OSR

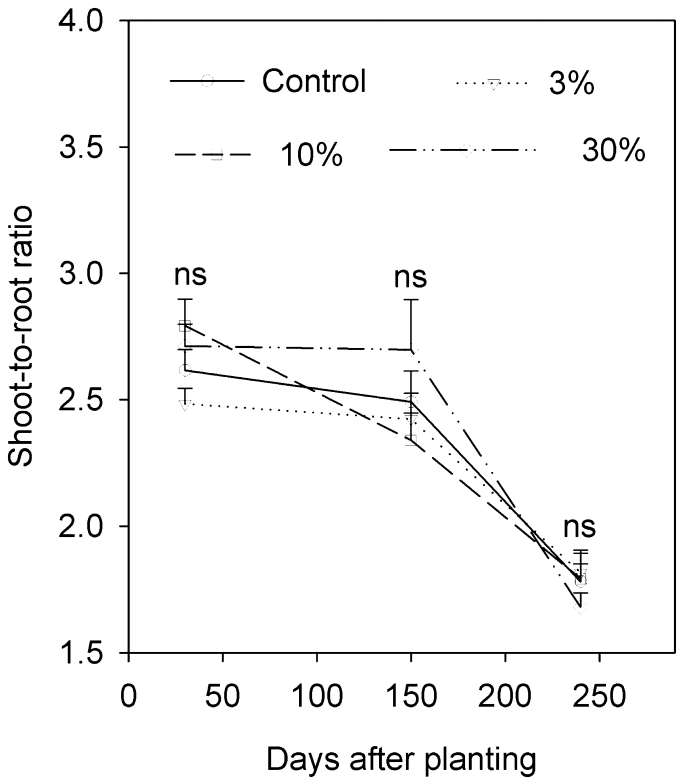

Fig. 4. Changes in shoot-to-root ratio of olive grown under different fresh OSR treatments. Data are means ± SE ($n = 6$). Means with different letter(s) on the same day are significantly different at $P < 0.05$.

treatment, and 9.2% for the 3% OSR treatment. In contrast, the fine root electrolyte leakage in the control plants showed a different trend, being minimal at the beginning and then increasing to the end of the experimental period (Fig. 5c).

The fine root respiration for all of the OSR treatments showed its maximum and minimum at 30 and 150 days, respectively, from the beginning of the experiment, and increased with the increasing OSR treatments. In particular, the fine root respiration for the 30% OSR treatment was significantly higher for the first two consecutive uprooting dates (after 30 and 150 days). At the end of the experimental period all of the OSR treatments showed similar fine root respiration (Fig. 5d). The correlation between the fine root respiration and the fine root relative growth rate was negative after 30 days, and completely disappeared after 240 days (Fig. 6).

3.4. Total chlorophyll content and chlorophyll a fluorescence

The decomposition of the OSR had no significant effects on chlorophyll content and maximum quantum efficiency of PS II at the leaf level (Table 3). For all of the OSR treatments and uprooting dates, the quantum efficiency of PSII (expressed as F_v/F_m) was >0.80. Instead, the total chlorophyll content decreased with increasing OSR for the first uprooting period, with a SPAD value of 85.9 ± 2.88 in the control and 81.3 ± 0.99 for the 30% OSR treatment, and reached a similar SPAD value at the end of the experimental period.

3.5. Analysis of growth substrates, leaves and fine roots

The TOM, TOC, and TN of the growth substrates increased with the increasing OSR treatments on each uprooting date (Table 1). However, this increment in TOM and TOC reached the maximum (62%, mean of TOM and TOC) and the minimum (17%, mean of TOM and TOC) at the beginning and end of the experimental period,

Table 3
Effects of fresh OSR decomposition on total chlorophyll contents and chlorophyll *a* fluorescence of the olive leaves.

Uprooting time(days)	OSR (%)	SPAD units	F_v/F_m
30	Control	85.9 ± 2.9	0.83 ± 0.01
	3	84.1 ± 2.2	0.82 ± 0.02
	10	82.4 ± 3.4	0.83 ± 0.01
	30	81.3 ± 1.0	0.81 ± 0.0
150	Control	84.1 ± 4.2	0.83 ± 0.01
	3	84.0 ± 4.2	0.83 ± 0.01
	10	83.4 ± 3.7	0.83 ± 0.01
	30	82.3 ± 3.9	0.82 ± 0.01
240	Control	83.3 ± 3.9	0.81 ± 0.01
	3	84.8 ± 3.0	0.83 ± 0.01
	10	83.5 ± 2.0	0.83 ± 0.01
	30	82.5 ± 2.7	0.83 ± 0.01

Data are means ± SE ($n = 6$).
OSR: olive shoot residues, F_v/F_m: PSII maximum efficiency

respectively, whereas the TN increment was maximum (117%) after 150 days from planting, and minimum (29%) at the beginning of the experimental period for the 30% OSR treatment, as compared to the control. Similarly, TOM and TOC of the control were increased with time by 31% and 24%, mean of TOM and TOC, after 30 and 240 days of decomposition compared to initial day of TOM and TOC of the control, respectively. At the beginning of the experimental period, the C/N ratio increased up to a maximum with the 10% OSR treatment, and then decreased. For the consecutive two uprooting dates, (after 30 and 150 days), the C/N ratio decreased with increasing OSR treatments. And at the end of the experimental period, the highest and lowest C/N ratios were recorded for the control and the 30% OSR treatments, respectively (Table 1).

A high polyphenols content was recorded for the fresh/undecomposed OSR (858 μg/kg). Thereafter, the addition of the OSR increased the polyphenols content directly in proportion to the treatments, for each of the uprooting dates. At the same time, the polyphenol contents of each OSR treatment decreased with incubation period, and reached a minimum at the end of the experimental period (Table 1).

For the first two consecutive uprooting dates, the fine-root and leaf N contents were inversely correlated with the growth substrate N content, which increased with increasing OSR treatments. At the end of the experimental period, the fine-root and leaf N contents were directly proportional to the N contents of the growth substrate ($r^2 = 0.87$, 0.90, respectively). At the same time, the shoot and fine root relative growth rate were inversely correlated with the growth substrate N concentration after 30 and 150 days, and directly proportional after 240 days of growth under the OSR treatments (Table 4, Fig. 7). The shoot and fine root P contents were not significantly affected by either the OSR treatments or the decomposition time (Table 4).

4. Discussion

Roots can detect (and react to) not only the abundance of soil resources, but also the presence of allelopathic substances with detection mechanisms that involve nontoxic and/or toxic signals.

This study shows that incorporation of high concentrations of OSR close to the roots of potted olive plantlets resulted in prolonged autotoxic effects on the fine root proliferation and a robust relationship between the increasing OSR treatments and the organic matter, and N and polyphenols contents of the growth substrate (Fig. 3, Tables 1 and 2). Besides, the increase with time of the organic matter content in the control showed that the increase in TOM and TOC in other treatments was not only due to OSR but also

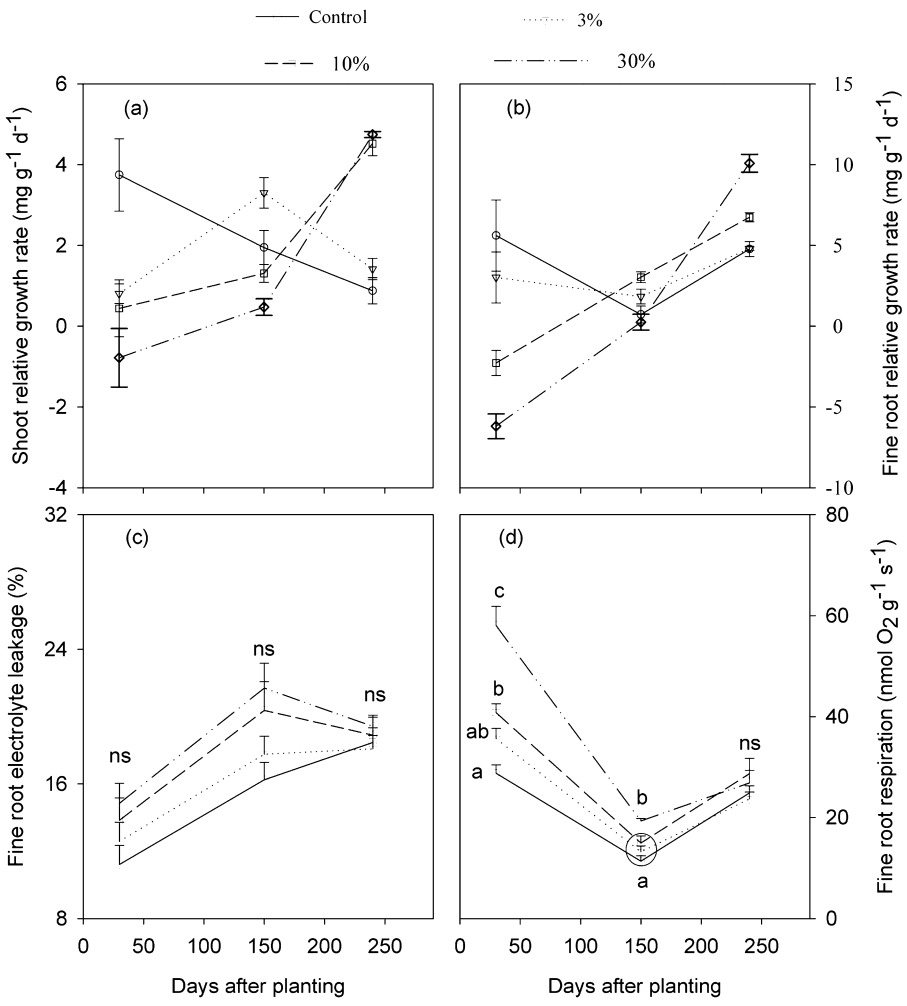

Fig. 5. Changes in patterns of shoot relative growth rate (a), fine root relative growth rate (b), fine root electrolyte leakage (c), and fine root respiration (d), under different fresh OSR treatments. Data are means ± SE ($n = 6$). Means with different letter(s) on the same day are significantly different at $P < 0.05$.

decomposition of the peat. Similar results are reported by Giorgi et al. (2010) when olive root growth was checked in 10% (v/v) olive dry husk incorporated into the basic peat-vermiculite-sand substrate. The decrease with time of C/N ratio with increasing concentration of OSR (Table 1) was probably due to the low C/N of OSR, respect to the growth substrate, and couple with lower rate of nitrogen mineralization compared to carbon loss. Similarly, Moradi et al. (2014) and Endeshaw (2013) reported lower C/N ratio during the decomposition of different oil palm and two-phase olive mill pomace, respectively. Apart from the prolonged effects on fine-root proliferation, the addition of OSR above 3% (v/v) to the substrate temporally inhibited shoot growth, stimulated transmigrating root growth, and induced lenticels development on the stem base, and the small and coarse roots in these olive cultivars. Similar

Table 4
Nutritional status of leaf and root of olive plantlet grown under different OSR treatments.

Uprooting time (days)	OSR (%)	Leaf N (%)	Fine root N (%)	Leaf P (%)	Fine root P (%)
30	Control	2.03	1.30	0.27	0.20
	3	1.60	1.07	0.31	0.26
	10	1.40	0.95	0.21	0.19
	30	1.26	0.97	0.20	0.21
	*SE	±0.17	±0.08	±0.03	±0.02
150	Control	1.75	1.09	0.21	0.21
	3	1.52	1.01	0.26	0.37
	10	1.42	1.00	0.28	0.37
	30	1.36	1.00	0.24	0.26
	*SE	±0.04	±0.02	±0.01	±0.04
240	Control	1.02	0.91	0.17	0.19
	3	1.18	0.93	0.17	0.37
	10	1.24	0.96	0.25	0.42
	30	1.51	1.11	0.23	0.35
	*SE	±0.10	±0.04	±0.02	±0.05

* SE: standard error of the four treatments.
OSR: olive shoot residues, N: nitrogen, P: phosphorus.

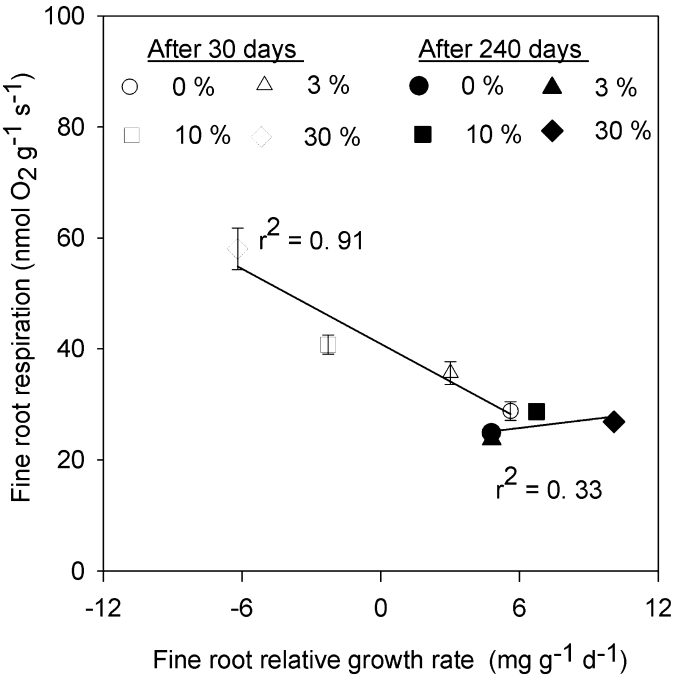

Fig. 6. Correlations between fine root respiration and fine root relative growth rate after 30 days and 240 days of growth under different OSR treatments.

autotoxic effects were registered on peach seedlings by Tagliavini and Marangoni (1992) when peach root residues were incorporated to the growth substrate. The inhibition of fine root growth for high OSR treatments was due to both autotoxic injury of the existing fine roots, and changes in the plant strategy in terms of the allocation of most of the respiratory energy to the transmigrating roots, in their search for new niches. In the field, for traditional and intensive olive farming systems, the production of transmigrating roots to avoid a toxic zone is probably a cost-effective strategy, although it will not be a cost-effective mechanism for high-density olive farming, where the availability of roots and the OSR-free zone is limited, or not available at all. On the other hand, production of transmigrating roots with higher OSR treatments maintained the total root mass for all of the uprooting dates above the initial total root mass. This compensated the loss of fine-root mass due to the antagonistic effects of the OSR decomposition, as well as maintaining a comparable shoot-to-root ratio to that of the control (Fig. 4).

The inhibition of fine root growth with the increasing OSR treatments was further confirmed with the increased root electrolyte leakage (Fig. 5), for both tested cultivars, by eroding the fine root cell membrane integrity and consequently affecting the plant nutrient absorption. On the contrary, high rates of fine root respiration but negative (decaying) fine root relative growth rates for the high-dose OSR treatment after 30 days of growth (Figs. 6 and 7), indicated that OSR decomposition in the early stages not only alters the fine root growth, but also partitions the respiratory energy. This might

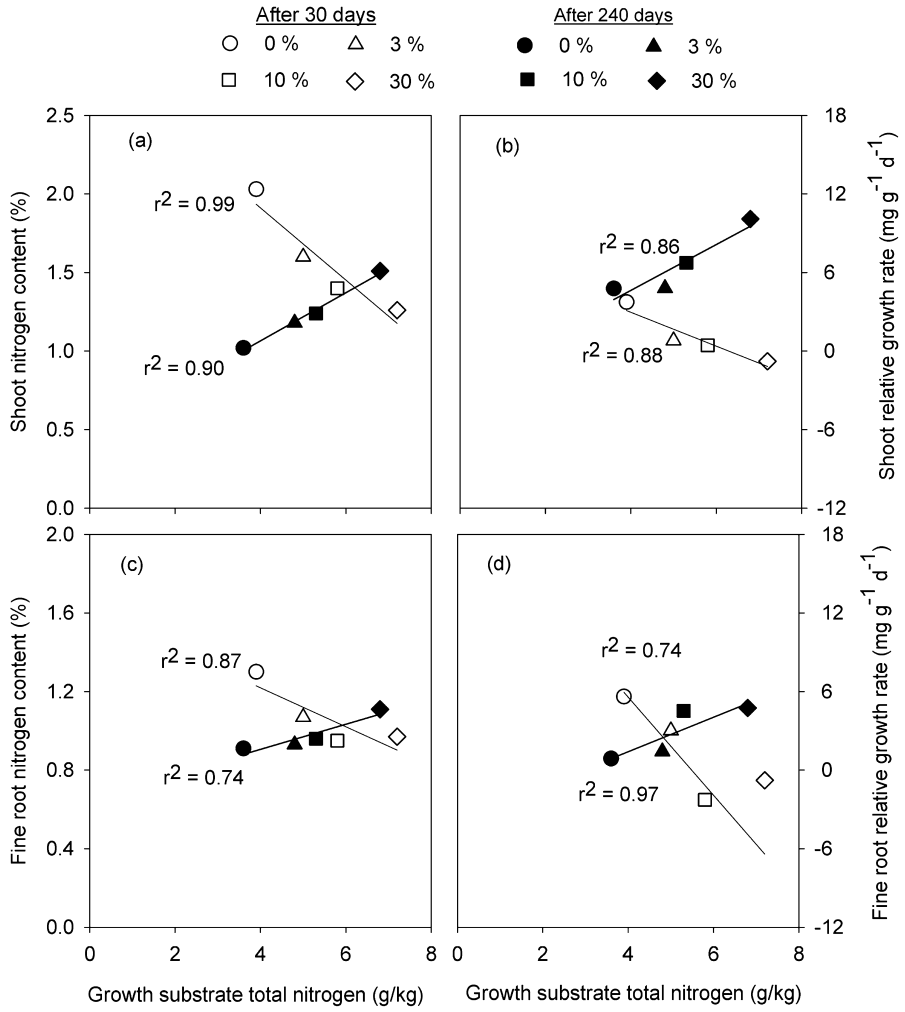

Fig. 7. Correlations between shoot nitrogen content (a), shoot relative grow rate (b), fine root nitrogen content (c), and fine root relative growth rate (d), with growth substrate nitrogen content after 30 and 240 days of growth in fresh OSR.

be due to the higher expenditure of respiratory energy for nutrient acquisition.

In this study, the development of lenticels during the first 150 days and the complete absence at the end of the experimental period suggest a temporal scarcity of oxygen, which will be mainly due to the increased water-holding capacity of the growing media with the increasing OSR treatments, and partly due to the decomposing aerobic microorganisms. Bonanomi et al. (2006) reported that anaerobic conditions augmented the autotoxic effects of litter decomposition on plant root growth by increasing the persistence/durability of the phytotoxic compounds produced during the decomposition.

Apparently, the transitory decrease in the leaf total chlorophyll content, the photochemical efficiency of photosystem II (F_v/F_m), and the foliar N content, while the total N increased with increasing OSR treatments above the 3% limit (Tables 3 and 4), was likely due to the reduced capacity of the plants to assimilate the available N because of the phytotoxicity of the OSR decomposition. Direct relationships between leaf N content and chlorophyll content, and leaf N content and F_v/F_m have been previously reported in *Acer pseudoplatanus*, *Quercus robur* and *Fagus sylvatica* (Percival et al., 2008).

Beyond the 3% (v/v) threshold of the added OSR, where autotoxicity has been reported to be absent or minimal in olive, these potted plantlets could no longer prevent autotoxicity from phytotoxins released during the decomposition of the OSR. In the field (in the Mediterranean region), and in particular in high-density olive farming, the coincidence of the onset of root growth and litter decomposition at the beginning of spring (Lodolini et al., 2011) coupled with the reduction in avoidance of autotoxicity of olive at high OSR levels, may exacerbate this problem. Therefore, incorporation of OSR beyond this 3% limit can have temporary effects on the olive growth by inducing morphological, physiological, and metabolic changes to the roots, and particularly to the fine roots.

5. Conclusions

Over the last decades, traditional olive orchard management has undergone significant changes, due to shift to the new olive plantation systems (intensive and super-high density olive orchard). This transition involved a general reduction of biodiversity of the organic residues in the orchard and a higher sedentary of the root system, so that possible autotoxic effect on olive growth production need to be studied. From the data obtained in the present study, we can conclude that the application of OSR increases soil fertility, TOM and C and total N, but it initially induces autotoxic effects on root and shoot growth. Therefore in the field, to increase synergic effects and avoid competition and toxicity of OSR on root and shoot growth and to improve soil fertility, it is necessary to know the exact quantity of OSR that is left on the field, for each type of olive orchard, and when and how to apply it. Such results cannot be extrapolated directly to field conditions, but this study supplies baseline information to understand the effects of increasing OSR doses on the olive growth and in particular on morphological, physiological, and metabolic changes to the root system. Further field experimentations are required to identify proper OSR application thresholds to increase soil fertility and avoid toxic effects on growth and production according to planting density, environmental conditions and cultivation management.

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