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Agronomic Exploitation of Olive Oil Mill Wastewater: Effect on Growth and Yield of Field Crops and Impact on Soil Fertility

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ABSTRACT

In Mediterranean countries, olive oil mills generate a liquid waste that could represent a potential source of pollution or an agronomic opportunity if recycled as fertiliser within the agrosystems. Hence, the effects of applying increasing amount of this effluent (0, 8, 16, 32 l m^{-2}) on growth and productive response of a series of field crops (forage sorghum, faba bean, durum wheat, berseem, malting barley, sulla meadow) as well as on the main soil properties were studied. The research was conducted in southern Italy using lysimeters as plots. The results highlighted that with the exception of faba bean, which experienced phytotoxicity with deleterious effects at higher rates of the effluent, the other tested crops were not penalised and responded positively in terms of plant survival, growth and yield as the waste supply increased within certain limits. Therefore, each crop reached the best productive performance with different level of residue. However, the favourable rainfall regime during the growing season of the crops played a key role. At soil level, changes emerged between the start and the end of the experiment for the analysed features, except those for pH and EC. Overall, the organic pool of the soil was relatively preserved, whereas an enhancement occurred for C.E.C. and for macronutrients, mainly P and K. The micronutrient levels also varied differently. However, the final variations of the soil characteristics were not always univocally sustained by the different waste rates. In conclusion, there are real possibilities of agronomic reutilisation of the waste in cropping systems typical of southern Italy, but others research into farming studies are needed to acquire a better evaluation of the environmental impact.

Keywords: durum wheat, Egyptian clover, faba bean, forage sorghum, sulla meadow, two-rowed barley

INTRODUCTION

Olive groves have long been a key component of Mediterranean agriculture. At present, Mediterranean countries provide over 98% of the world's annual olive oil production, which totals approximately 3.1×10^6 Mg (FAOSTAT Agriculture 2008), and Italy is the second major producer after Spain (almost 25 and 40% of the total, respectively). Nevertheless, the connected processing activities of olive oil mills (OOM), which require water during certain operative steps, release an aqueous residue, known as olive mill wastewater (OMW), which is often difficult to manage. Its quantity and composition varies widely in relation to a number of pedoclimatic, agronomic and technological conditions (Kapellakis *et al.* 2008). The latter seem to be the major determinant, given that the average volume of the effluent has been estimated to be equal to *ca.* 0.25 and 2 l Kg^{-1} of milled olives as the adopted extraction process is, respectively, the modern continuous two- and three-phase centrifugation. On the other hand, liquid waste can attain up to 0.50 l Kg^{-1} of processed raw material when the traditional discontinuous scheme of cold-pressure is used. Nowadays, unlike Spain and Croatia where the new two-phase centrifuge-type OOM are widespread, either the three-phase centrifuge-type and pressure-type OOM remain prevalent in other olive oil producers' Mediterranean countries, including Italy (Paris 1998; Tamburino *et al.* 1999; Kapellakis *et al.* 2008). According to a prudential estimate, based on the global dimension of olive oil industry, between 10 and 30×10^6 m^3 of OMW, which in Italy accounts for about 2×10^6 m^3, needs to be managed annually considering that, from a compositional point of view, such residue represents a potential source of pollution if unrestrainedly dispersed in the environment (Spandre and Dellomonaco 1996; Rana *et al.* 2003; McNamara *et al.* 2008). The concentration of residual lipids (0.1-29.8 g l^{-1}), phenols (0.4-14.3 g l^{-1}) and salt (EC 6.5-7.5 mS cm^{-1}), the acidic reaction (pH 4.5-5.9), the values of COD (15.2-389.5 g l^{-1}), BOD_5 (17.0-134.8 g l^{-1}) and BOD_5/COD (generally below 0.5) of waste, variously contribute to the unpleasant smell with methane emission, the transitory harmful effects towards a range of living organisms, and to the biodegradation resistance of it (Paris 1998; Sierra *et al.* 2007). Precisely, assuming a unit value of 45-55 kg BOD_5 Mg^{-1} of olives for the polluting organic load, irrespective of the extraction system, Boari *et al.* (1984) estimated equal to 2800-3600 Mg BOD_5 day^{-1} the whole polluting potential of OOM in Mediterranean countries. These aspects form the main constrains in the ecological removal of by-product requiring complex and expensiveness technological treatments of detoxification or bioremediation strategies, although the risk of environmental contamination related to the heavy metals content and the health hazard in comparison to municipal wastewater are both normally lower (Rinaldi *et al.* 2003; Komilis *et al.* 2005; Sierra *et al.* 2007; McNamara *et al.* 2008). Alternatively, considering the natural biodegradative capacity of soils and the chronic deficit of organic carbon, nutrient and water availability in the Mediterranean environment, OMW could constitute an opportunity to regulate the fertility and water-balance if it is correctly recycled in view of a *restitutio ad integrum* within the agro-system. Specifically, it is possible to exploit the notable amounts of organic matter (14-18%), phytonutrients, mainly potassium (630-5000 mg l^{-1}), nitrogen in organic form (140-1106 mg l^{-1}) and phosphorus (42-915 mg l^{-1}), and water (83-97%) of the waste as a fertilizer and irrigation resource (Cabrera *et al*. 1996; Paris 1998; Sierra *et al.* 2007; Kapellakis *et al.* 2008).

Because of the common small dimension of OOM and

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their dispersal in an extensive uneven territory, the Italian oil-millers retain easiest and relatively inexpensive the direct land application of OMW. Therefore, this option has been extensively practised especially in southern Italy, subject to agreement with farmers, often using unsustainable rates of waste matter (up to 360 m^3 ha^{-1} y^{-1}), until the introduction of legal limits. In accordance with the precaution principle in the framework of European Union wastewater policy (EU Council Directive 271/1991 and successive amendments), the being Italian legislation (Law 574/1996) imposes specific restrictions in the controlled on field disposal of this liquid by-product. Amongst other thinks, the law fixes rigidly the maximum quantitative threshold, equal to 50 and 80 m^3 ha^{-1} y^{-1}, respectively for the OMW derived from pressure and centrifuge system, as well as its storage time of up to 30 days (Tamburino *et al.* 1999). Unfortunately, the regulations in force are not only dissimilar among the various olive mill-producing countries but also contradictory between sites of a given country (Kapellakis *et al.* 2008). At the present, thus it is reasonable to think that the waste is often illegally disposed in the environment.

Since the final decade of the last century, a number of bibliographic references have been recognised beneficial or only transitory negative effects on various physical, chemical and biological properties of the soil as well as on the crops performances' spreading untreated, pre-treated or composted OMW in the short and medium-term (Bonari *et al.* 1993; Proietti *et al*. 1995; Cabrera *et al.* 1996; Cegarra *et al.* 1996; Demicheli and Bontoux 1996; Cox *et al*. 1997; Cardelli *et al.* 1998; Vassilev *et al.* 1998; De Simone *et al*. 1999; Garcia-Ortiz *et al*. 1999; Paredes *et al.* 1999; 2005; Sierra *et al.* 2001; 2007; Casa *et al.* 2003; Rinaldi *et al.* 2003; Cereti *et al.* 2004; Kotsou *et al.* 2004; Mekki *et al.* 2006; Brunetti *et al.* 2007; Cayuela *et al* 2007; El Hassani *et al*. 2007; Altieri and Esposito 2008; Belaqziz *et al.* 2008; Di Serio *et al.* 2008; Kapellakis *et al.* 2008; López-Piñeiro *et al.* 2008; Mechri *et al.* 2008; Ouzounidou *et al.* 2008). In brief, the review of the above cited literature reveals that, in general, using OMW under conditions of moderately alkaline reaction, adequate cation-exchange capacity, hydraulic conductivity and clay level, $CaCO_3$ and organic matter contents of the soil it is possible to obtain the highest agronomic effectiveness with the lowest risk of groundwater pollution, especially in a semi-arid Mediterranean environment. In particular, it has been stated that the amendment with this agro-waste, throughout the regulation of the aggregates stability and porosity of the soil can favourably affect the mobility of certain herbicides and attenuate leaching and toxicity. Others protective and conservative functions of the residue on bare soil exercising an attenuation of the raindrop impact and consequent runoff, as well as a reduction of moisture loss have been considered. Sometimes, it has also been described an unfavourable evolution of nitrification process in the short period after the application of the effluent on soil with a delay in nitrate availability, even tough an agronomic exploitation of this effect as occurs for the slow-release nitrogen fertilizer has been suggested. A potential of OMW for long-term advantageous changing of soil microbial communities' organization and functionality has been highlighted, particularly to nitrogen-cycle bacteria, and otherwise it has been proposed the use of it against certain soil-borne pathogens and weeds because of its inhibiting and hallelopathic actions. Furthermore, a positive influence, though not always univocal owing to the interactive effects with environmental (soil and climate) and genetic (species and cultivars) factors, have been reported on growth and productivity of different crops using fresh or differently pre-treated OMW. However, short-term toxicity has also been noticed, besides on the rhizosphere microbiota, in different crops depending mainly on the dose and cumulative effect, time (phenological phase) and space (distance from plants) application of this unconventional water, which have been recurrently considered the foreseeable upshot of its definite and restricted availability period during the autumn and winter seasons.

Hence, owing to the soil-crop-atmosphere system complexity, an agreement has not been reached as to whether the use of OMW is sustainable from an agro-ecological point of view, especially through long-term repeated spreading on the same site.

Bearing in mind the aforementioned restrictions and potentialities of agro-industrial waste, knowledge of the application upper limit for each of the species planned within a given field crop sequence and the understanding of the long-term effects on soil-crop systems represent critical agronomic issues in a correct on-farm local management of it. For this reason, the effects of supplying increasing doses of OMW on growth and yield of field crops typical of southern Italian cereal-forage cropping system as well as the overall impact on soil fertility were the subject of assessment in research carried out over a multi-year period.

MATERIALS AND METHODS

Experimental site and lysimeter system

The experiment took place during the period 2001-2007/08 in Calabria, the 2nd important olive-region of Italy characterised by a significant incidence of small sized OOM. Trials were carried out at Gallina (38°10′N, 15°45′E, 232 m a.s.l), Reggio Calabria, a location with a typical Mediterranean climate, from hot-temperate to semi-arid. The soil of the study site is a 'Typic Haploxeralfs' (USDA). It has a sandy-clay-loam texture and contains through the 0-80 cm profile 56.8, 14.6 and 29.0% of sand, silt and clay, respectively, referred to fine fraction. Moreover, the soil is of non-calcareous nature (1% total $CaCO_3$), non-saline (EC 0.3 mS cm^{-1}) and has sub-alkaline reaction (pH 7.6), low content of organic matter (1.1%), total-N (0.33‰), assimilable-P (0.06 mg kg^{-1} P_2O_5) and assimilable-K (63,6 mg kg^{-1}), C/N rather high (18.5), as well as low cationic exchange capacity (11.3 meq 100 g^{-1}) and exchangeable sodium percentage (9.0 %).

At first, within a pre-prepared experimental area of 1600 m^2 (40 m × 40 m), a set of 12 unfilled containers of 1-m^3 (1 m × 1 m × 1 m) were placed in the soil, 5 m apart, aligned N-S, to implement an unsheltered lysimeter installation. The border of the containers was kept 10 cm above the ground level to reduce the boundary layer effect. After the positioning of a thin layer of draining material and a non-woven fabric, a PVC access tube (diameter 12 cm, length 1 m) was inserted into each lysimeter to recuperate leached liquid. Subsequently, the containers were filled with soil comes from a neighbouring area previously managed uniformly, respecting its original profile. In order to avoid soil water-saturation and stagnancy in the lysimeters, excess leachate was extracted, if necessary, by using a pump after the application of OMW or after heavy rainfall. It was sampled and stored for later analysis.

Field crops, OMW treatments and lay out

During the research period, six crops were grown sequentially in the lysimeters managed as plots according to the following temporal plan: 1st year, forage sorghum sudangrass (*Sorghum bicolor* L. Moench ssp. *drummondii*, Nees ex Steud., de Wet & Harlan); 2nd year, faba bean (*Vicia faba* minor L.); 3rd year, durum wheat (*Triticum durum* Desf.); 4th year, Egyptian clover or berseem (*Trifolium alexandrinum* L.); 5th year, two-rowed barley or malting barley (*Hordeum distichum* L.); 6th and 7th years, sulla meadow (*Hedysarum coronarium* L.). The species were chosen taking into consideration the representativeness in southern Italian cropping systems and the possibility of intercept a period of fresh OMW availability. The lysimeters were surrounded by a buffer area cultivated with the studied species to minimize the interference of adjacent unused areas. The fallow periods were uniformly managed by manually controlling weeds when necessary.

Taking into account the specification of Italian legislation, an explorative procedure was adopted, assessing for each studied crop besides an untreated control (T_0), the application of three OMW doses increasing in geometric progression (q=2) starting from the maximum legal amount: 8, 16, 32 l m^{-2} (T_8, T_{16}, T_{32}, respectively). Treatments were assigned in the lysimeters according

Table 1 Composition of olive oil mill wastewater (2001-2006/07). Average values and variability over six years (CV = coefficient of variation).

Feature	Mean	CV
COD (mg l^{-1} O_2)	36859.8	28.3
BOD_5 (mg l^{-1} O_2)	17967.9	52.3
pH (H_2O 1:2.5)	4.8	1.6
EC (mS cm^{-1})	7.3	6.8
Water (%)	98.7	2.6
Suspended solids (mg l^{-1})	8794.0	27.8
Dry matter (mg l^{-1})	7628.7	59.4
Residual lipids (%)	0.9	39.5
Phenols (mg l^{-1})	3.1	50.2
Organic N (mg l^{-1})	784.5	70.8
N-NH_4^+ (mg l^{-1})	65.8	56.2
N-NO_2^- (mg l^{-1})	0.6	53.4
N-NO_3^- (mg l^{-1})	7.2	50.4
Total P (mg l^{-1})	167.9	12.1
Total K (mg l^{-1})	3163.8	31.3
Total Ca (mg l^{-1})	138.3	46.2
Total Mg (mg l^{-1})	139.1	29.7
Total Na (mg l^{-1})	84.7	27.4
Total Fe (mg l^{-1})	81.4	44.6
Total Cu (mg l^{-1})	0.98	33.2
Total Pb (mg l^{-1})	0.05	12.1

to a completely randomised design with three replicates for the 1st crop, following the same arrangement for successive crops in order to evaluate the potential residual effect on the same soil with the same waste rate.

OMW characteristics and management, field measurements and samplings, analytical procedures

The effluent used in the experiment was purchased annually from the same OOM, located near the experimental site, which employs a three-phase centrifugation decanter for oil separation. It was previously sampled and analysed in duplicate by conventional methods (APAT & CNR-IRSA 2004), and the main characteristics are shown in **Table 1**.

Considering that the OMW availability period in the study site is approximately from November to March, application to the soil was soon done to avoid waste degradation, prior to the sowing of forage sorghum and at early stage of the growing season for the other crops. **Table 2** has extra information and details concerning agronomic management for each of the studied species.

Weather conditions were monitored at the farm level from an automated station consisting of a data logger (model CR10X, Campbell Science, UK) equipped with a complete set of standard meteorological sensors.

In order to assess the level of plant survival in response to waste application, the plant population of the studied species was verified by counting the number of plants/shoots on an undisturbed area per experimental unit. In each cropping seasons, representative sample of plants periodically collected on a measurement-area per plot, according to a scheme based on days after sowing (DAS), were analysed in terms of aerial dry matter and its partitioning, by means of oven drying at 80°C, and leaf area using a LI 3100 electronic area meter (Li-Cor Inc., Lincoln, NB, U.S.A.). With regard to the sorghum and clover crops, after the principal cycle, samplings to assess plant growth recommenced on the same area during successive regrowth cycles within the cropping season. For both forage crops, final dry matter taking from the plants outside the growth-sampling area of each plot by successive cuttings was used to calculate seasonal biomass yield, i.e. all the cuts. Precisely, cuts were performed for the two species at convenient stage based on plant height for sorghum (70-100 cm) and at blooming stage for clover. Considering the dry conditions during the growing season of sorghum, irrigation water (50 l m^{-2}) was supplied at sowing and after the successive three cuts to favour plant regrowth. The 2nd regrowth cycle of clover was destined to seed production as occurs normally in the cropping environments of the south of Italy. For sulla meadow, which was maintained for a biennium, a single cutting for hay per year was carried out at the onset of the flowering stage, outside the growth-sampling area, to evaluate seasonal forage yield. Seed yield and related components (mature reproductive structures) of grain crops (faba been, wheat and barley) were assessed from plants of apposite harvest-area of plots.

The whole changing of the soil physico-chemical and chemical features were evaluated from initial (before the start of the research) and final (after the conclusion of the research) samples taken by a core drill in each lysimeter throughout the profile up to a depth of 80 cm, split into 0-40 and 40-80 layers. Samples were analysed after air-drying, crushing and 2-mm sieving, in accordance with the official procedures (Italian law "Ministero delle Politiche Agricole, Alimentari e Forestali" 1992 - 1999). Particularly, the following methods were adopted: pH, potenziometric; $CaCO_3$ total and active, De Astis and Droineau, respectively; EC, saturation paste extract at 25°C; organic matter, Walkley-Black; total N, Kjeldahl; assimilable P and K, Olsen and Merwin Peach, respectively; total S and assimilable B, UV visible spectrometry; C.E.C., NH_4 acetate; exchangeable cations (K, Ca, Mg, Na) and micronutrients (Cu, Zn, Mn, Fe), atomic absorption spectrometry.

Data analysis

Because the experimental conditions varied annually, data were processed separately. For each species, the evolution of growth based on unit of area was described using the functional approach proposed by Hunt (1982). Yield data expressed in terms of seasonal dry biomass or grain per unit of area depending on the crop-type were plotted against OMW rates with the intent of identifying the best-fitting dose-response relationships in a descriptive way. For the other productive traits and soil features it was not always possible to establish a definite trend in response to OMW doses, therefore data was subjected to appropriate one-way ANOVA in accordance to the GLM model of the CoStat 6.003 programme (CoHort Software, 1998-2001, Monterey, USA). The means of the treatments were compared by the least significant difference test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Meteorological conditions

The weather at the experimental site throughout the research period was substantially consistent with average long-term conditions and the year-to-year variability of

Table 2 Cultural and methodological details regarding the research.

Crop	Forage sorghum	Faba bean	Durum wheat	Egyptian clover	Two-rowed barley	Sulla meadow
Growth season	2001	2002	2003	2004	2005	2006/07-2007/08
Cultivar	'Grazer N' hybrid	'Sikelia'	'Valbelice'	'Lilibeo'	'Scarlett'	'S. Omero'
Sowing date	24 April	8 January	15 January	20 February	24 January	16 November
Seeding rate	40 m^{-2}	45 m^{-2}	400 m^{-2}	1500 m^{-2}	390 m^{-2}	819 m^{-2}
Sowing type	rows, opportunely spaced					
OMW supply time	pre-sowing	3-4 leaf	tillering	4-5 leaf	tillering	3-4 leaf (1st year only)
OMW supply date	27 March	18 March	18 March	7 April	25 March	7 March
Growth sample	plants on 200 cm^2	2 plants	5 plants	plants on 150 cm^2	5 plants	plants on 240 cm^2
Weed control	manually, if necessary					
Water regime	irrigated (200 l m^{-2})	rainfed				
Cuttings	4		-	2 (+ seed)	-	2 (1st + 2nd year)

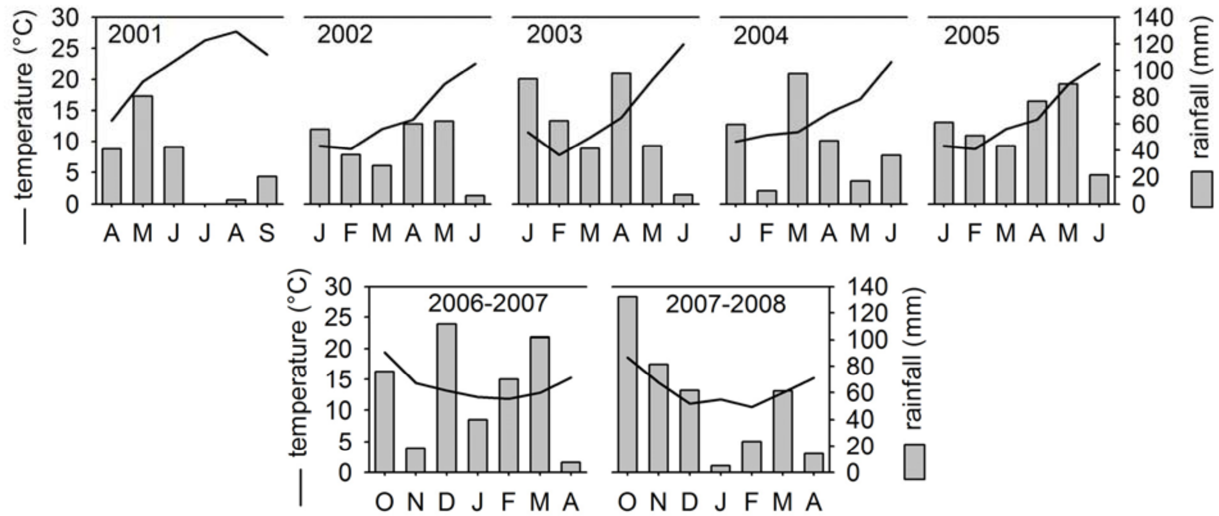

Fig. 1 Weather throughout the research period. Monthly average temperature (line) and total rainfall (bars).

mean air temperature and rainfall was within the typical range (**Fig. 1**).

In the 1st year (2001), for the duration of the growing season of sorghum, the temperature ranged between 13.4°C in April and 27.7°C in August followed by a gradual decrease to 24.0°C in September. Rainfall was 157 mm from May to September, most of which was concentrated in May and June (55 and 29%, respectively). Therefore, supplemental water by irrigation was required from the time of sowing, although 41 mm of rain fell in April. From the 2nd to 5th year of research (2002-2005), during the period January-June corresponding respectively to the growing season of faba bean, durum wheat, Egyptian clover and two-rowed barley, temperature increased in 2002 (from 9.6 to 24.1°C) and 2004 (from 10.0 to 22.8°C). In contrast, in both 2003 and 2005, temperature decreased (from 11.5 to 7.8°C and from 9.3 to 8.8°C, respectively) between January and February, then increased until June (25.7 and 22.5°C, respectively). During most of the spring in 2003, 2004 and 2005, temperature was lower in comparison to that recorded throughout the same season of 2002. In the same four years (2002-2005), rainfall was 249, 345, 267 and 343 mm, respectively from January to June. Despite the differences in terms of total amount, the distribution of rain during the cycle of the four crops, excluding June, was generally regular in the 2nd, 3rd and 5th year, while in the 4th year there was less rainfall in February and May. In the final period of research (2006-2008), when sulla meadow was grown, there was a fairly mild thermal regime, with the exception of the intermediate ten days of December 2007 and February 2008. Rainfall from October to April was 425 and 379 mm in 2006-07 and 2007-08, respectively. In the last cropping season, rainfall between end of winter and start of spring was limited.

Growth and yield of forage sorghum (1st year of the cropping sequence)

The plant population verified at the end of the principal cycle of sorghum was unaffected by OMW application (44 plant m^{-2}, average of treatments), almost certainly because it preceded the sowing. As suggested by Bonari *et al.* (1993) this agronomic option is successfully practicable for spring field crops. Indeed, the inhibition of germination and seedling emergence with a negative impact on the number of plants per unit of area is mainly due to the phenolic com-

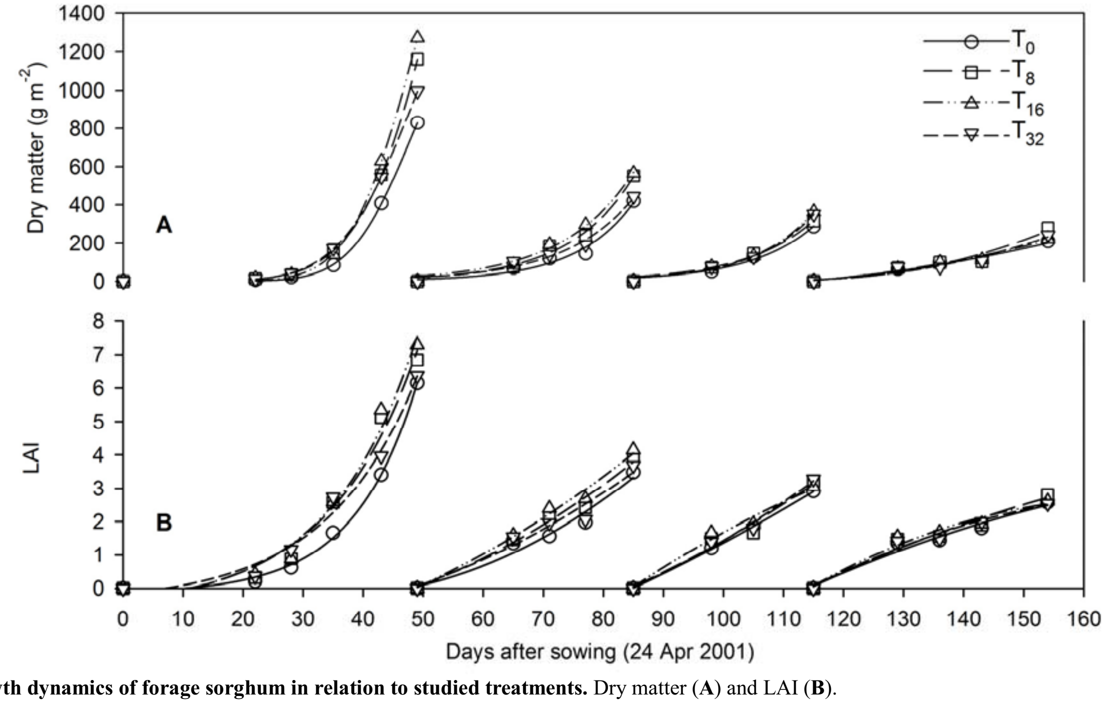

Fig. 2 Growth dynamics of forage sorghum in relation to studied treatments. Dry matter (**A**) and LAI (**B**).

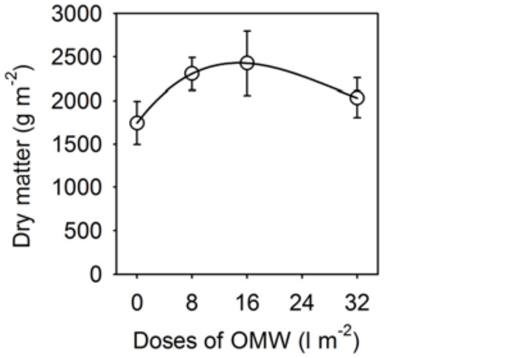

Fig. 3 Seasonal biomass yield of forage sorghum vs. OMW doses (2001). Vertical bars indicate the standard deviation of the mean (n=3).

pounds present in fresh OMW (Komilis *et al*. 2005; Mekki *et al.* 2006).

In general, the growth dynamics of the species within each of the four monitored sampling series (principal growth cycle and 1st, 2nd and 3rd regrowth) followed an exponential-type evolution, with the maximum weight per unit area and LAI reached at the final measurements in correspondence with productive cuttings, 49, 85, 115 and 154 DAS, respectively (**Fig. 2A, 2B**). At this time, irrespective of treatments, dry weight and LAI of the crop diminished throughout the entire growing season, from 1065, to 495, 329 and 238 g m^{-2} and from 6.7 to 3.8, 3.3 and 2.6, respectively, between the principal growth cycle and the three successive regrowths. However, the decline of crop leaf area was slight compared to that of dry weight owing to the higher incidence of leaves in comparison with stems on the total crop dry matter in the 2nd and 3rd regrowth (not shown). Moreover, the growth curves of dry matter and LAI highlighted that the response of the crop to increasing OMW supply was not univocal. In particular, from the final dry matter data of the four growth cycles, it was possible to note a manifest difference between treatments in both the principal growth cycle and 1st regrowth compared with subsequent ones. In fact, in terms of dry matter accumulated per unit of area, at the end of the principal cycle the plants of T_8 and T_{16} attained higher weight than the control, while only the weight of T_{16} plants appreciably differed from that of T_{32} (830, 1160, 1273 and 997 g m^{-2}, correspondingly to the increasing OMW rates). The same T_8 and T_{16} evidenced higher weight of plants than those of other treatments in the 1st regrowth (419, 551, 567 and 443 g m^{-2}, correspondingly to the increasing OMW rates); in the 2nd one, the plants of T_8, T_{16} and T_{32} reached quite similar weight, which was greater than that of T_0 plants (286, 315, 364 and 351 g m^{-2}, correspondingly to the increasing OMW rates). Compared to the latter, in the final regrowth, only the weight of T_8 plants differed positively from those of other treatments (209, 279, 222 and 242 g m^{-2}, correspondingly to the increasing OMW rates). In terms of leaf extension, it was possible to observe that in general, in all the sorghum growth cycles, the increase of OMW dose caused only a tendential increment of LAI. Indeed, the enhancement of this index compared to control treatment progressively decreased from the first to the fourth sampling series reaching at most 1.2, 0.7, 0.4, 0.2 units, respectively.

Coherently with the behaviour of sorghum at each cut, the crop response to OMW input in terms of seasonal forage yield evidenced a considerable increase attaining 2429 g m^{-2} raising the supply of waste from 0 to 15 l m^{-2}; after this utmost there was a productive flexion less than proportional with the further increase of the effluent (**Fig. 3**).

Growth and yield of faba bean (2nd year of the cropping sequence)

Firstly, it is important to state that the higher tested doses of OMW resulted in the appearance of damage in faba bean

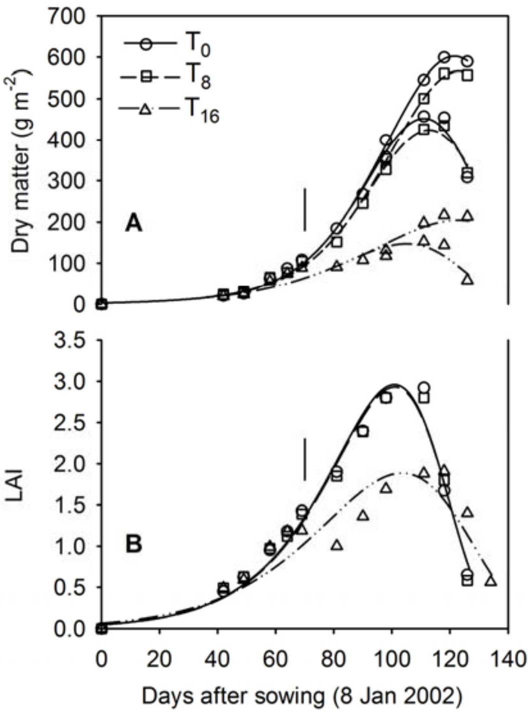

Fig. 4 Growth dynamics of faba bean in relation to studied treatments. Dry matter (**A** - the subdivision within each curve shows the increase of grain weight) and LAI (**B**). The vertical segments indicate the time of OMW supply.

plants. However, compared to untreated control (41 plants m^{-2}), while a diffuse chlorosis and necrosis of leaves and stems caused the death of all seedlings in the two weeks following the application of 32 l m^{-2} dose, only a decrease in plant population was observed in response to the 16 l m^{-2} dose (18 plants m^{-2}). Instead, the crop reacted well in terms of plant survival to the 8 l m^{-2} dose (40 plants m^{-2}), although it caused a reduction of growth and yield.

The change in dry matter during the cropping cycle evidenced a typical sigmoid-time course, despite different levels among treatments, principally because of the above-mentioned differences in the number of plants per unit of area (**Fig. 4A**). Indeed, at the end of the cycle, the dry matter per unit of area for T_0 and T_8 plants (589 and 557 g m^{-2}, respectively) did not substantially differ, while the T_{16} plants drastically reduced the accumulation of dry matter (213 g m^{-2}), besides for the lesser plant population, as a consequence of the lower final plant dry weight (12 g $plant^{-1}$ in T_{16} against 14 and 15 g $plant^{-1}$, respectively, in the T_8 and T_0 treatments).

In general, the LAI of the crop varied characteristically with time and the differences between treatments reflect those observed in terms of dry matter accumulation (**Fig. 4B**). LAI of T_0 and T_8 plants equally increased gradually reaching a peak of 2.9, on average, at 111 DAS, than it declined rapidly to a mean value o 0.6 at 126 DAS. The LAI of T_{16}, instead, initially increased similarly to the other treatments, but evidenced a temporary stop after the spreading of OMW reaching the value of 1.0 at 81 DAS; afterwards it increased again up to 1.9 at 118 DAS lessening to a final value of 0.6 measured 8 days later than the final sampling of T_0 and T_8 treatments. This depressive effect of 16 l m^{-2} OMW dose on LAI is not directly attributable to a lowering of leaf area per plant (not shown), but rather to the above-mentioned severe reduction of the plant population.

As the OMW supply increased the crop responded with a proportional decrease of grain yield from the highest amount of 281 g m^{-2} obtained by the untreated control, though the decrement did not exceeded 15% within the top limit of the residue admitted by Italian legislation (**Fig. 5**).

The yield decline of the crop may be partially attributa-

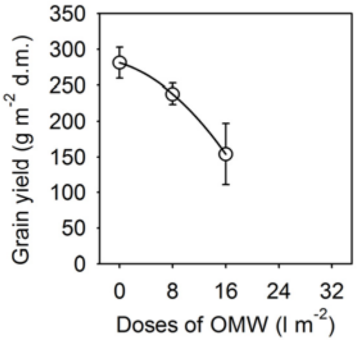

Fig. 5 Grain yield of faba bean vs. OMW doses (2002). Vertical bars indicate the standard deviation of the mean (n=3).

Table 3 Variations of yield components in faba bean, in relation to studied OMW treatments (T_{32}, unavailable). Values with different letters are significantly different at $P \leq 0.05$.

OMW treatment	Pods per plant (n)	Seeds per pod (n)	Seed weight (mg)
T_0	4.75 b	2.83 a	546.7 a
T_8	4.25 b	2.81 a	515.6 a
T_{16}	6.20 a	2.65 b	582.9 a

ble to the above cited plant population effect, given that the almost halved number of plants per unit of area for T_{16} treatment compared to that of control, led to a certain adjustment of the plant yield components (**Table 3**). Nevertheless, the increase in the number of pods and mean seed weight per plant, only slightly compensated for the lower number of plants m^{-2}, with limited influence on grain yield. In contrast, despite not resulting in a reduction in plant population, the dose of 8 l m^{-2} OMW had a negative effect principally in terms of pod numbers.

The performance of the species was consistent with other experimental results. In fact, De Simone *et al.* (1999) observed the absence of significant phytotoxic and genotoxic effects on faba bean plants grown in two different soils treated with OMW rate not superior to 50 m^3 ha^{-1}. On the other hand, Mekki *et al.* (2006) presented evidence that irrigation with untreated OMW at 100 m^3 ha^{-1} inhibited seed germination of the same species as a result of the high concentration in phenolic compounds and caused negative effects on its growth and yield. Nevertheless, considering the dose-dependent sensitivity of the species, in all probability the phytotoxicity was also worsened by the residual effect of waste applied in the first year of research.

Growth and yield of durum wheat (3rd year of the cropping sequence)

Durum wheat tolerated the spreading of OMW without particular consequences in terms of plant loss as seen by the lowest variation in the number of fertile shoots m^{-2} at harvest (415, 404, 407 and 417, respectively for T_0, T_8, T_{16} and T_{32}). A yellowing of the crop canopy, progressively more intense with the increase in the OMW dose was observed initially, but the symptom disappeared within few days. Rainfall after the application of waste almost certainly plays a crucial role in attenuating phytotoxicity.

The dry matter accumulation of the crop evolved in general according to a polynomial exponential-type curve (**Fig. 6A**). Plants reached the peak values of dry weight between 133 and 141 DAS: 968, 1191, 1118, 1010 g m^{-2}, according to the studied treatments series. During the subsequent period of development, a decline in dry matter occurred in terms of both whole and grain weight of the plants. The leaves senescence and shedding as well as the earlier activation of catabolic process of the stored substances in the kernels after physiological maturity, which often occurs with greater intensity under warm conditions of the Mediterranean environment, could explain this final loss of dry weight. Consequently, at the end of the crop cycle the total dry matter accumulated by the plants varied appreciably in relation to the different treatments increasing from 711 to 902 g m^{-2} between T_0 and T_8. In contrast, the two higher OMW doses resulted in its reduction up to 808 and 802 g m^{-2}.

The time course of LAI of the crop was typical and similar for all the tested treatments series (**Fig. 6B**). The index gradually increased until the heading stage at 105 DAS when the value measured for T_8 crop (2.7) was greater than that of the control crop (2.3) and the others (2.5, on average of T_{16} and T_{32}). Later, LAI declined similarly for all the treatments up to a final average value of 0.7.

Examination of the relationship in **Fig. 7** revealed that the grain yield versus OMW supply augmented appreciably up to an highest level of 314 g m^{-2} increasing the waste rate from 0 to a threshold 10 l m^{-2} and then diminished gradually maintaining from 26 l m^{-2} onwards almost asymptotically a value of about 270 g m^{-2}.

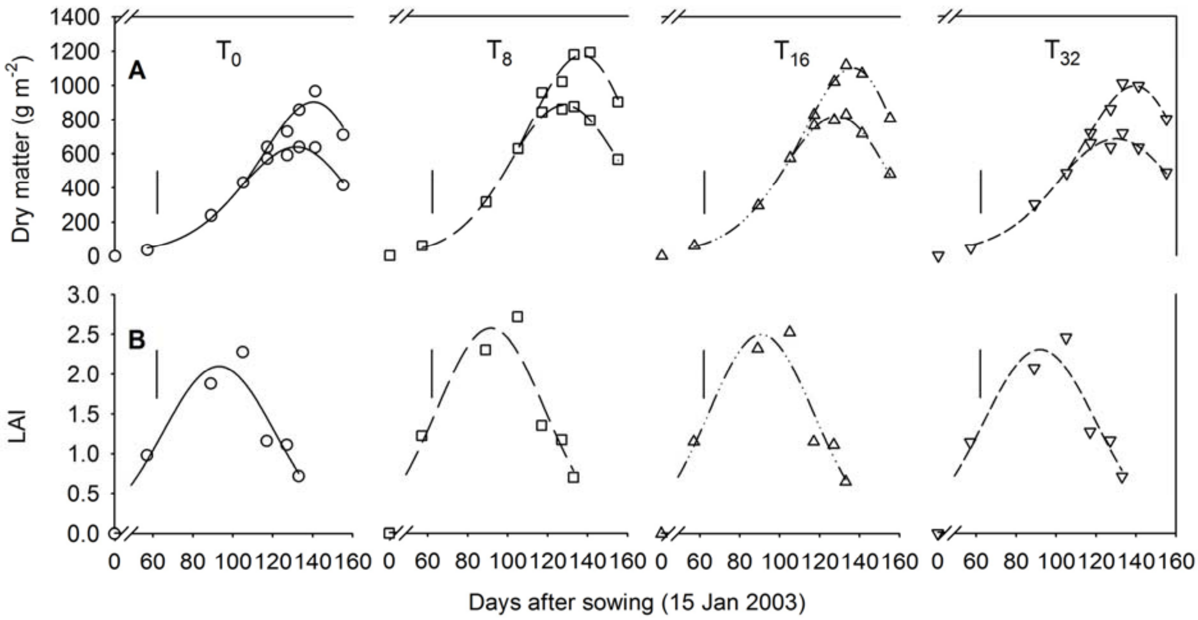

Fig. 6 Growth dynamics of durum wheat in relation to studied treatments. Dry matter (**A** - the subdivision within each curve shows the increase of grain weight) and LAI (**B**). The vertical segments indicate the time of OMW supply.

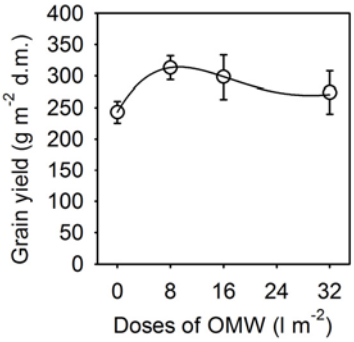

Fig. 7 Grain yield of durum wheat vs. OMW doses (2003). Vertical bars indicate the standard deviation of the mean (n=3).

Table 4 Variations of yield components in wheat, in relation to studied OMW treatments. Values with different letters are significantly different at $P\leq0.05$.

OMW treatment	Total spikelets per ear (n)	Fertile spikelets per ear (n)	Kernels per ear (n)	Kernel weight (mg)
T_0	16.94 a	12.11 b	20.22 b	38.09 c
T_8	17.17 a	12.75 a	21.58 a	38.70 c
T_{16}	16.28 b	12.00 b	18.83 c	40.20 b
T_{32}	16.92 a	11.83 c	19.50 bc	41.49 a

Of the crop yield components, the number of total spikelets per ear was lower for T_{16} compared to the other treatments assessed which reached a similar performance (**Table 4**). The number of fertile spikelets per ear and the number of kernels per ear were both significantly greater for T_8 compared to those ascertained for the other treatments, whereas kernel weight significantly increased from T_0 to T_{32}.

The behaviour of the crop substantially confirms previous findings reported in the literature. Rinaldi *et al.* (2003) applying 50 t ha^{-1} of OMW highlighted a moderate toxicity with a certain decrease of plant population and some necrosis of younger leaves of wheat plants, which however responded differentiating greater number of secondary shoots growing and yielding without significant difference compared to an untreated control. Mekki *et al.* (2006) found that in the same species, seed germination was strongly inhibited and growth and productivity were negatively affected by irrigation with 100 m^3 ha^{-1} of fresh OMW. Brunetti *et al.* (2007) also evidenced a significant increase in durum wheat straw, grain yield and some other productive traits as the soil was amended with lagooned and catalytically digested OMW at rates of 300 and 600 m^3 ha^{-1}.

Growth and yield of Egyptian clover (4th year of the cropping sequence)

This forage leguminous species did not show any trace of phytotoxicity caused by the application of OMW, as demonstrated also by the plant population, which at the end of the principal cycle was substantially unaffected by the different treatments (899, 931, 877 and 925 plant m^{-2}, in the order of the studied treatments).

Unlike forage sorghum, the growth of berseem expressed by dry matter accumulation and expansion of leaves overall the treatments was rather similar in quantitative terms between the two monitored sampling series (principal growth cycle and 1st regrowth) (**Fig. 8A, 8B**). In particular, during the principal cycle both dry matter per unit of area and LAI assumed a sigmoidal-type evolution with a limited initial increase, which became progressively higher in the intermediate period and than diminished again. In contrast, the time course of both dry weight and LAI of the plants was of exponential-type, but practically linear, for the most part of the 1st regrowth cycle. Therefore, within the sampling series the highest weight per unit area and LAI was reached at the final measurements 78 and 99 DAS, in occasion of the productive cutting for the main cycle and 1st regrowth, respectively. At these times, irrespective of the treatments, the dry matter per unit of area was comparable between the principal cycle and the 1st regrowth (on average, 248 and 261 g m^{-2}, respectively) as well as the LAI of the crop, which reached an average value equal to 5.0 for both sampling series.

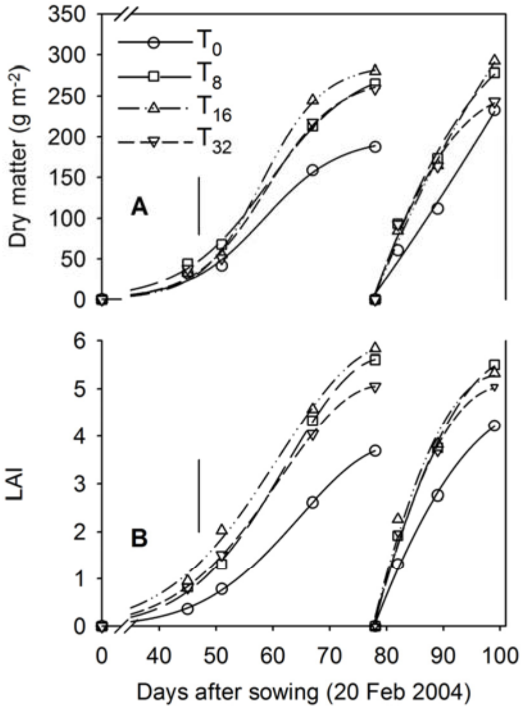

Fig. 8 Growth dynamics of Egyptian clover in relation to studied treatments. Dry matter (**A**) and LAI (**B**).

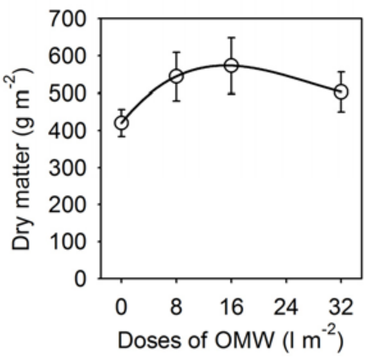

Fig. 9 Seasonal biomass yield of Egyptian clover vs. OMW doses (2004). Vertical bars indicate the standard deviation of the mean (n=3).

The supply of different doses of OMW caused significant variations in the growth level of Egyptian clover. In fact, at the end of the main cycle, the dry weight per unit of area and LAI of T_0 plants (187 g m^{-2} and 3.7) were lower compared to those reached by the other treatments. No relevant difference between T_8 (265 g m^{-2} and 5.6) and T_{16} (280 g m^{-2} and 5.8) plants was observed, but the values of the latter treatment exceeded those of T_{32} (259 g m^{-2} and 5.1). At the end of the 1st regrowth, the plants of T_0 and T_{32} evidenced similar dry matter per unit of area (233 and 243 g m^{-2}, respectively), but different LAI (4.2 and 5.0, respectively), although inferior to those of T_8 and T_{16}, (279 and 292 g m^{-2}, and 5.5 and 5.3, respectively) which were analogous.

Because of the productive performance of berseem at each cut, the dose-response relationship of **Fig. 9** shows that the seasonal forage yield of the crop increased with raising

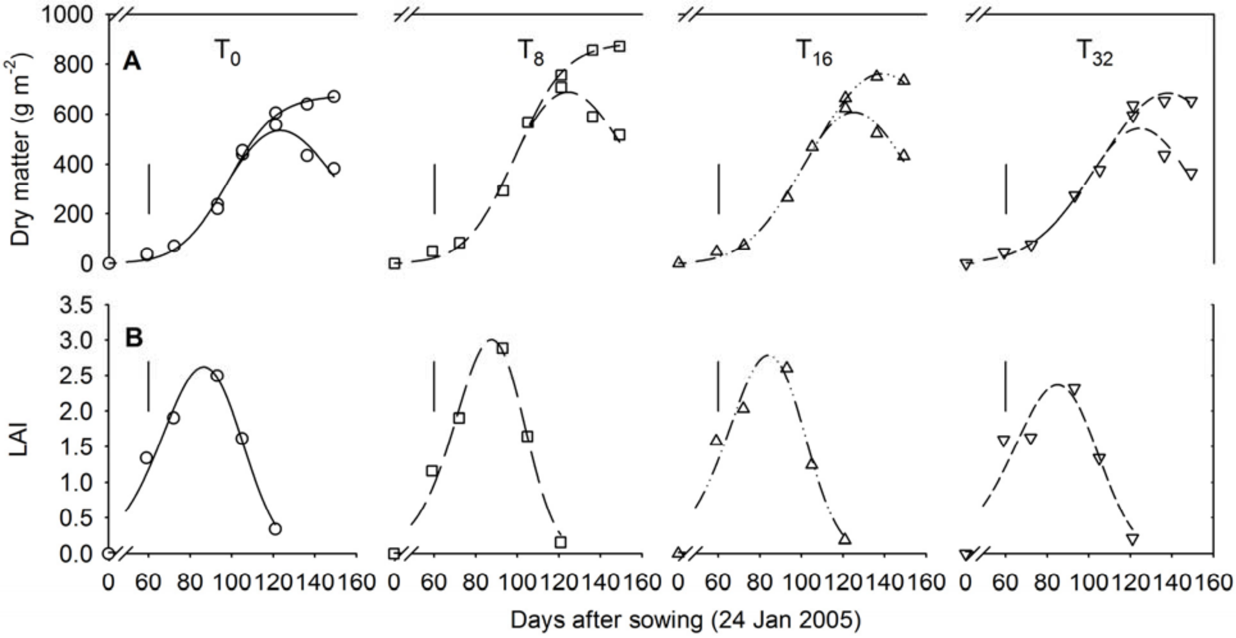

Fig. 10 Growth dynamics of two-rowed barley in relation to studied treatments. Dry matter (**A** - the subdivision within each curve shows the increase of grain weight) and LAI (**B**). The vertical segments indicate the time of OMW supply.

the waste rate reaching a peak of 573 g m^{-2} applying 16 l m^{-2} OMW, although over this highest limit a moderate productive lessening occurred.

Regarding the 2nd regrowth of the crop destined to seed production (not shown), all three OMW doses caused an analogous general depression in the level of total dry matter accumulated compared to that of untreated control (244.1 g m^{-2}, on average, against 274.1 g m^{-2}) at the end of the growth period (145 DAS). Considering only the seed weight, instead, various differences without any particular trend between treatments were evidenced (62.8, 76.1, 69.1 and 84.7 g m^{-2}, in the order of studied OMW rates).

Growth and yield of two-rowed barley (5th year of the cropping sequence)

Like durum wheat, malting barley tolerated the spreading of OMW without difficulty evidencing a high survival level with a non-relevant variation in the number of fertile shoots per unit of area at harvest (352, 340, 344 and 340, respectively for T_0, T_8, T_{16} and T_{32}). Most likely, similarly to other studied winter cereal, the rainfall during the period after the application of waste contributed to the dilution of it avoiding any inhibitory effect for the crop as suggested also by the findings of Mekki *et al.* (2006). In addition, according to Bonari *et al.* (1993), irrespective of doses, no harmful effect is expected for barley if there is an adequate time, at least 60-day, between the sowing and the application of waste.

The growth and development of barley was similar to that of wheat, with the exception of a lower mean quantitative level attained by the dry biomass and the extension of leaves, although the maximum LAI was slightly higher (**Fig. 10A, 10B**). Apart from the time course, compared to the wheat, the upper LAI of barley occurred earlier at 93 DAS (2.5, 2.9, 2.6 and 2.3, in the order of the OMW doses) and it may be partially attributable to its earliness, whereas the highest dry matter level was late between 136 DAS and the end of the cycle (149 DAS). Moreover, there was a less prominent final reduction of the dry weight per unit of area in barley than in the other studied cereal.

At maturity stage, an appreciable increase was observed in accumulated total dry matter of the plants from the untreated control to 8 l m^{-2} of OMW, from 672 to 872 g m^{-2}, and a gradual reduction in biomass weight up to 735 and 655 g m^{-2} supplying the crop, respectively, with 16 and 32 l m^{-2} of the waste.

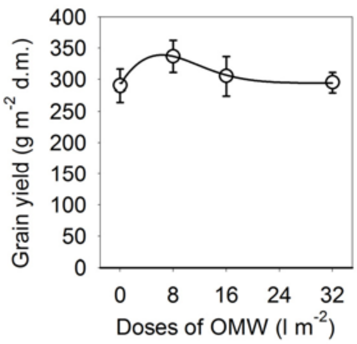

Fig. 11 Grain yield of two-rowed barley vs. OMW doses (2005). Vertical bars indicate the standard deviation of the mean (n=3).

Table 5 Variations of yield components in barley, in relation to studied OMW treatments. Values with different letters are significantly different at $P \leq 0.05$.

OMW treatment	Total spikelets per ear (n)	Kernels per ear (n)	Kernel weight (mg)
T_0	21.28 c	19.08 b	43.46 b
T_8	24.13 a	21.83 a	47.04 a
T_{16}	22.82 b	21.42 a	45.76 ab
T_{32}	22.67 b	21.17 a	44.27 ab

Comprehensibly, the curve fitted to seed yield versus the waste rate, evidenced that there was a steady increase of the amount of grain up to a maximum of 339 g m^{-2} raising the OMW dose from 0 to an upper limit of 7 l m^{-2} and as the effluent increased again there was a gradual productive reduction (**Fig. 11**). Similarly, Rinaldi *et al.* (2003) affirm that, like other winter cereals, also for barley there was no negative effect caused by the application of fresh OMW with doses not exceeding 80 m^3 ha^{-1}, but they instead noticed a benefit in terms of crop biomass yield due to the fertilization effect of waste.

Analysis of the productive traits pointed out that the plant fertility was lower for T_0 than that for the other treatments. While the number of total spikelets was significantly greater only for T_8 compared to the other treatments, the number of fertile spikelets per ear, which for this cereal corresponds to the number of kernels per ear, was lower for T_0 in comparison to those similar measured for the other treatments (**Table 5**). Mean kernel weight, instead, evi-

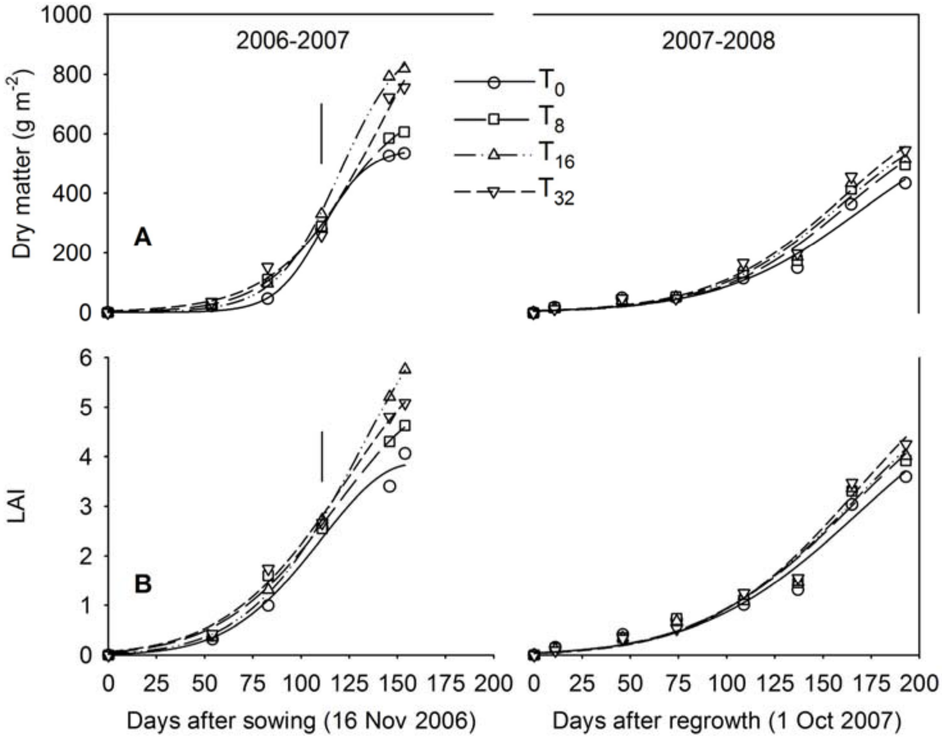

Fig. 12 Growth dynamics of sulla in relation to studied treatments. Dry matter (**A**) and LAI (**B**) in the two years of productive cycle.

denced a significant increase from T_0 to T_8 followed by a decrease in response to the other two treatments, which had a kernel weight similar and undifferentiated from both the preceding.

Growth and yield of sulla (6th and 7th year of the cropping sequence)

In accordance with the traditional agronomic model adopted in southern Italy, sulla crop was maintained over a two-year period managing the meadow in order to perform a single productive cut for hay at the beginning of flowering stage of either the principal cycle (2006/07) and the regrowth cycle after the autumnal vegetative resumption (2007/08).

The number of plants per unit of area verified at the end of the main cycle of sulla (332, 327, 340 and 307 plant m^{-2}, in ascending order of the waste dose) revealed the reaction ability of the species towards the application of OMW since an early stage of growth as observed for Egyptian clover. Anyway, the markedly lower plant population compared to that expected on the bases of the adopted seeding rate was usual for the species sown in autumn-winter period under Mediterranean environments. It has been attributed either to the incidence of seed-coat dormancy or unfavourable thermal conditions during the initial phase of growth as well as to various combinations of these factors (Anastasi and Santonoceto 2000). Therefore, the modest variation in the number of plants m^{-2} between the untreated control and other studied treatments corroborates this inference.

In general, the growth dynamics of the species in terms of dry matter and leaf extension during the main cycle was of an exponential or sigmoidal-type with minimal increase in the initial period which became gradually greater in the intermediate phase and than decreased again (**Fig. 12A, 12B**). In the 2nd year, after the vegetative resumption, which occurred earlier due to the consistent and regular autumnal rainfall, the sulla plants regrew with a time-evolution similar to that of the preceding year, but more slowly and constantly at inferior level especially during winter, almost certainly as a result of the suboptimal thermal regime. As observed for the other studied forage crops, within each of the two productive cycle of sulla, the maximum weight per unit of area and LAI was measured at the final sampling, respectively, 154 DAS in the 1st year and 193 days after resumption (DAR), when the productive hay-cuttings were carried out. In correspondence to this final measurement, because of the different degree of growth between the two years, irrespective of the treatments, the dry weight per m^{-2} and the LAI in the regrowth cycle of the meadow were, respectively, 181 g m^{-2} and 1 unit lower than in the principal cycle. However, after the OMW supply, the plants varied their growth in response to the rising dose, and concluded the main cycle reaching 534, 607, 819 and 756 g m^{-2} of dry matter and 4.1, 4.6, 5.8 and 5.1 of LAI. In any case, there was a slight difference between T_0 and T_8, T_{16} and T_{32} for dry biomass and between T_8 and T_{32} for LAI.

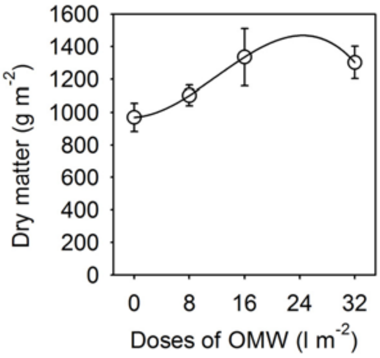

Fig. 13 Seasonal biomass yield of sulla meadow vs. OMW doses (2006-07 and 2007-08). Vertical bars indicate the standard deviation of the mean (n=3).

Conversely, during the regrowth cycle, the variations among the studied OMW doses were attenuated and hence in terms of either dry matter or LAI of the meadow a difference was finally observed (193 DAR) only compared to the untreated control (519 g m^{-2} and 3.7, on average of T_8 T_{16} and T_{32} against 435 g m^{-2} and 4.0 of T_0).

From the curve interpolated to seasonal dry biomass produced by sulla meadow with two cuts against the waste rate it was possible to deduce that the amount of forage progressively increased reaching a peak of 1467 g m^{-2} increasing the OMW dose from 0 up to a threshold of 25 l m^{-2} and than the yield began decreasing (**Fig. 13**).

Overall change of the soil characteristics

Details about the variation of the physico-chemical and chemical characteristics in the 0-40 and 40-80 cm of soil profile between the beginning and the end of the research are shown in **Table 6**.

First, it is important to point out that the variations of the initial level of the analysed features were quantitatively negligible considering the entire profile of the soil subjected to the studied treatments. Consequently, even though some significant differences within each examined layer emerged for total-$CaCO_3$, total-N, assimilable P and K, C.E.C. and single exchangeable-cations, E.S.P. and various micronutrients, the soil may be considered substantially homogeneous at the start of the experiment. On the contrary, compared to the initial conditions, the soil data at the end of the research denote various changes, which were relevant only for some of the examined features, but not always directly attributable to the level of OMW input since the control treatment behaved in a similar way.

Particularly, in contrast to the findings of short-term studies, in spite of its acidic reaction the waste did not affect the pH of the soil, which was found essentially unvaried, almost certainly because of the effective action exerted by the buffer-power during the prolonged duration of the research. Cabrera *et al.* (1996) obtained similar result in a medium-term trial (3 years) conducted in an uncultivated calcareous-clayey soil. Therefore, the carbonates content of the experimental soil, which although lowest diminished further at the end of the experiment, as well as the adequate percentage of mineral colloids, doubtless played a certain role as pH stabilizing, given that the total $CaCO_3$, especially for T_8 and T_{16}, evidenced greater difference compared to the initial values in each examined layer.

Final values of soil salinity expressed as EC were equal or lower than the starting ones, with major discrepancy for T_{16} in both layers. Only within the upper layer, final EC of T_{32} was significantly higher in comparison to that of control and other treatments. Presumably, the amount of natural (rainfall) and supplementary (irrigations in the 1st year) water intercepted during the long experimental period, which was often particularly consistent after the OMW application, had a dilution-effect successfully contributing to remain the soil EC below the risk level for salinization.

A lower final organic matter content was found in the whole soil profile with respect to the starting level, but as the OMW supply increased, the differences compared to the initial state was progressively attenuated in the upper layer. Conversely, there was slighter discrepancy between the initial and final soil sampling for all treatments in the inferior stratum. Nevertheless, in the 0-40 cm profile T_{16} had significantly higher content of organic matter compared to T_8 and T_{32}, and to T_0, whereas below 40 cm depth both T_{16} and T_{32} had similar greater organic matter level. The results

Table 6 Changes of physico-chemical and chemical features in two layers (0-40 and 40-80 cm) of the soil profile before the start and after the end of the experiment (B and A, respectively), in relation to studied OMW treatments. In each row and within each layer, values with different letters are significantly different at $P \le 0.05$.

OMW treatment		T_0	T_8	T_{16}	T_{32}	T_0	T_8	T_{16}	T_{32}
Feature	Soil layer	0-40 cm				40-80 cm			
pH	B	7.4 a	7.5 a	7.5 a	7.6 a	7.5 a	7.5 a	7.8 a	7.7 a
H_2O (1:2.5)	A	7.7 a	7.6 a	7.8 a	7.7 a	7.8 a	7.8 a	7.8 a	7.8 a
$CaCO_3$ total	B	0.65 b	1.15 a	1.40 a	0.61 b	0.56 b	1.02 a	1.12 a	0.69 b
(%)	A	0.30 a	0.30 a	0.30 a	0.30 a	0.30 a	0.30 a	0.30 a	0.30 a
$CaCO_3$ active	B	0.30 a	0.80 a	0.55 a	0.10 a	0.10 a	0.65 a	0.11 a	0.36 a
(%)	A	0.25 a	0.12 a	0.12 a	0.12 a	0.19 a	0.19 a	0.19 a	0.18 a
EC	B	0.39 a	0.39 a	0.50 a	0.55 a	0.38 a	0.40 a	0.42 a	0.41 a
(mS cm^{-1})	A	0.33 b	0.36 b	0.35 b	0.46 a	0.38 a	0.38 a	0.33 a	0.41 a
Organic matter	B	1.16 b	1.47 a	1.86 a	1.14 b	0.78 a	0.82 a	0.89 a	1.01 a
(%)	A	0.66 c	1.07 b	1.68 a	1.04 b	0.64 b	0.62 b	0.75 a	0.86 a
Total N	B	0.21 b	0.21 b	0.87 a	0.37 b	0.07 b	0.42 a	0.41 a	0.08 b
(‰)	A	0.15 c	0.38 b	0.89 a	0.40 b	0.22 c	0.49 b	0.45 b	0.65 a
Assimilable P	B	0.06 a	0.06 a	0.07 a	0.06 a	0.07 a	0.05 b	0.07 a	0.06 b
(mg kg^{-1})	A	22.5 b	65.0 a	57.0 a	63.5 a	38.0 c	58.0 b	65.0 a	69.0 a
Assimilable K	B	76.0 a	53.0 b	69.5 a	64.5 a	63.5 a	64.0 a	59.0 a	59.0 a
(mg kg^{-1})	A	94.0 d	160.0 b	134.5 c	256.5 a	83.0 c	127.5 a	105.5 b	111.0 a
Total S	B	438.4 c	600.5 b	742.9 a	544.7 b	763.8 a	696.9 a	726.3 a	585.5 a
(mg kg^{-1})	A	620.9 b	660.3 b	567.2 b	823.7 a	717.5 b	775.0 a	660.2 b	806.3 a
C.E.C.	B	14.2 a	11.6 a	7.4 b	11.0 a	13.0 a	12.2 a	10.1 a	10.8 a
(meq 100 g^{-1})	A	17.5 a	13.9 b	13.5 b	14.2 b	15.7 a	16.1 a	14.9 b	14.2 b
Exchangeable K	B	0.20 a	0.14 a	0.18 a	0.17 a	0.17 a	0.17 a	0.15 a	0.15 a
(meq 100 g^{-1}).	A	0.24 c	0.41 b	0.27 c	0.66 a	0.21 b	0.33 a	0.23 b	0.29 ab
Exchangeable. Ca	B	9.69 a	8.01 a	4.10 b	7.06 a	8.55 a	8.00 a	6.24 a	7.07 a
(meq 100 g^{-1})	A	12.76 a	9.55 b	9.58 b	9.30 b	10.97 a	11.34 a	10.89 a	9.94 b
Exchangeable Mg	B	0.83 c	0.71 c	0.96 b	1.02 a	1.13 a	1.02 a	0.96 a	0.86 a
(meq 100 g^{-1})	A	0.60 b	0.67 b	0.73 b	0.94 a	1.17 a	0.77 b	0.69 b	0.81 b
Exchangeable Na	B	1.13 a	0.85 b	0.90 b	0.94 b	1.02 a	0.99 a	1.05 a	0.90 a
(meq 100 g^{-1})	A	1.00 a	0.91 a	0.67 a	0.98 a	0.77 a	0.99 a	0.61 a	0.83 a
E.S.P.	B	7.9 b	7.9 b	12.2 a	8.5 b	7.8 b	8.4 b	10.7 a	8.6 b
(%)	A	5.7 a	6.6 a	4.9 a	6.8 a	5.2 a	6.1 a	4.6 a	5.9 a
Assimilable Cu	B	11.32 b	11.53 ab	12.06 a	10.69 c	10.34 b	10.85 b	11.52 a	10.36 b
(mg kg^{-1})	A	3.21 a	3.46 a	3.46 a	3.88 a	1.77 b	3.16 a	3.51 a	3.40 a
Assimilable Zn	B	2.76 ab	2.69 b	2.82 a	2.58 c	2.66 a	2.32 b	2.49 ab	2.29 b
(mg kg^{-1})	A	1.15 a	1.27 a	1.02 a	1.28 a	0.93 a	1.15 a	1.09 a	1.12 a
Assimilable Mn	B	99.44 b	100.07 b	103.56 a	97.56 b	90.90 b	89.95 b	96.16 a	89.67 b
(mg kg^{-1})	A	1.16 b	5.31 a	2.78 b	2.66 b	1.45 b	2.76 a	2.70 a	1.22 b
Assimilable Fe	B	90.18 b	96.63 a	98.88 a	93.51 a	79.44 a	85.74 a	85.43 a	85.68 a
(mg kg^{-1})	A	3.52 c	4.38 b	4.74 b	5.79 a	1.97 b	3.50 a	3.75 a	3.92 a
Assimilable B	B	0.34 a	0.35 a	0.36 a	0.35 a	0.33 a	0.32 a	0.35 a	0.30 a
(mg kg^{-1})	A	0.45 b	0.92 a	0.50 b	0.82 a	0.48 b	0.87 a	0.51 b	0.58 b

were expected, taking into account the combination between the initial deficit of organic matter and the high percentage of sand in the soil, the warm climatic conditions and the long period of research, which was rather favourable to the mineralisation processes. Indeed, an enrichment of soil organic matter in response to waste application has been commonly observed in short-term experimental conditions (Kapellakis *et al.* 2008). However, Cabrera *et al.* (1996) using elevated repeated doses of OMW across a 3-years period (up to 1830 l m^{-2}, in total) in a soil poor of organic matter ascertained an increase in the latter to a greater extent in the 0-50 cm rather than in 50-100 cm part of the profile. Therefore, under conditions of limited fertility as in the present case, the role of the waste in preserving the soil organic matter can be particularly precious.

In contrast, the final level of total-N increased differently apart from the reduction observed for the untreated control in the upper soil layer. Nonetheless, the highest difference between initial and final soil sampling was observed for T_8 and T_{32}, respectively, in the 0-40 and 40-80 cm of the profile. OMW supply determined an increase in total-N content, which was significantly greater for T_{16} and T_{32} in the order of the considered soil depth. The increase in total-N level may be justified in part by the enrichment in N_2-fixing bacteria stimulated by the presence of waste, as affirmed Cabrera *et al.* (1996). Moreover, the observed final increase of total-N suggests a probable modulating influence of immobilisation process, which involves pH and/or inhibiting compounds of phenolic nature that contrasted the rapid mineralisation of the organic N. This type of evolution is explainable also by the mean values of C/N above 17 and avoided an excessive concentration of nitrate with possible risk of their leaching and a consequent groundwater contamination. In fact, the very low N-NO_3 content found in the drainage liquid periodically sampled by the lysimeters (not reported) corroborates this inference recurrently described in the literature (Cardelli and Benitez 1998; Sierra *et al.* 2007). On the other hand, the risk of this type of pollution seems to be least in the semiarid environments of southern Italy due to the high evaporative demand, which occurs in the three-four months subsequent to OMW spreading, as well as to the adequate clay content of soils (Proietti *et al.* 1995). In the present experiment, a positive role in the N availability of soil can also be attributed undoubtedly to the impact of the adopted cropping system, which included forage and grain leguminous species.

Assimilable-P content was markedly enhanced in each soil layer for all the OMW treatments compared to the untreated control at conclusion of the experiment, although the increase compared to the initial level was greater for T_8 and T_{32} (0-40 cm) and for T_{16} and T_{32} (40-80 cm). Moreover, OMW application at all the rates in the upper layer, as well as at the two higher rates in the deeper stratum improved significantly the concentration of this macronutrient in comparison to the other tested treatments.

An analogous effect was observed for assimilable-K content, which enhanced greatly especially in the upper soil horizon where it significantly increased also with the increase of the wastewater doses, whereas in the inferior horizon, the enrichment of this macro-element prevailed for the lowest and the highest doses compared to the other treatment and control. An improvement of P and K availability of the soils generally related to the richness of the OMW used has been widely documented in the literature (Cabrera *et al.* 1996; Sierra *et al.* 2007; Kapellakis *et al.* 2008; Mechri *et al.* 2008).

As regards to the total-S, the high content measured before the start of the experiment was maintained at the end of it also in the untreated soil. Nevertheless, a significant enhancement of the concentration of this anion was determined by the waste applied at highest rate in the two examined soil layers, as well as in response to T_{16} treatment in the deeper stratum.

C.E.C. and the proportion of both exchangeable K and exchangeable Ca were generally higher at the end of the trial period than at the beginning in each of the two examined soil layers. However, irrespective of the observed enhancement, the final variations of these features were not properly attributable to the OMW treatments except that for exchangeable K, which in particular was significantly greater for T_{32} and for the latter and T_8 treatment, respectively, in the strata 0-40 and 40-80 cm. It is relevant highlights that exchangeable-Ca, like in the soil sample taken at the starting of the experiment, in spite of the strong enrichment in assimilable-K, prevailed in the exchangeable complex with respect to the main other cations for all the studied treatments, with possible beneficial effect for physical properties of this soil. However, because of the general exchangeable-Mg reduction occurred between the initial and finishing soil sample, the final Ca/Mg increases accentuating the relative disadvantages for Mg availability except for T_{32} (9.9 against 16.2, on average of the other treatments in 0-40 cm layer) and T_0 (9.4 against 14.3, on average of the other treatments in 40-80 cm layer). The Mg/K also decreased, but overall maintained an acceptable level (between 1.7 for T_8 in upper layer and 5.6 for T_0 in deeper layer), which does not imply excessive antagonism between the two cations and consequent problem in their relative availability. In any case, our data concerning the exchangeable complex, contrast with the findings of Cabrera *et al.* (1996) who recognized minimal variation of C.E.C. together with a decrease of exchangeable-Ca and a slightly increase of exchangeable-Mg after three year of experimentation.

Regarding the exchangeable-Na, which was found to be relatively stable, only a slight increase for T_8 and T_{32} emerged in the upper soil layer at the conclusion of the experiment. Moreover, in general, the final level of E.S.P. was lower than the initial one, and it diminished to a greater extent for T_{16} treatment in the two examined soil strata. In any case, from the start of the experiment E.S.P. of the soil had values below the threshold of sodification hazard.

With reference to the set of the five main micronutrients analysed, which were initially all sufficiently represented in each of the two examined soil horizons, a generalised considerable decrease was evidenced as the experiment was concluded for assimilable Cu, Zn, Mn and Fe, whereas assimilable B increased. Surprisingly these changes presented approximately the same magnitude for all the studied treatments in each explored soil layer and thus seem to be unrelated to the waste factor. Some significant advantage due to the OMW application emerged at the end of the trial period for Mn, Fe and B. Because of the limited concentration or lack of these elements in the oil mill effluent and the absence of reintegration by means of fertilizers during the experiment, such micronutrients depletion of the soil is explainable by the crop removal, excluding other possible loss.

CONCLUSION

Multi-year research concerning the influence of OMW on growth and yield of different field crops in sequence and on soil fertility, although not yet decisive, make available results applicable to the operative conditions of Mediterranean semiarid prone environments where olive growing and the connected processing activity often coexist with cereal-forage cropping models in an integrated agro-industrial system.

Forage sorghum, faba bean, durum wheat, berseem, malting barley and sulla meadow, were subjected to OMW rates, starting from the highest legally permitted in Italy (8 l m^{-2}) and exceeding this limit once and two-fold (16 and 32 l m^{-2}), together with an untreated control, to explore the feasibility of exploiting the residue to a greater extent than at present.

The species studied gave a varied response to OMW demonstrating different abilities in terms of reaction and survival to the potential phytotoxicity, renewal of the growth and productive performance owing partly to time of waste application and meteorological conditions.

In particular, the supply of OMW before sowing does not appear to have caused damage to sorghum, which on the contrary evidenced a positive "dose effect" reaching the highest seasonal forage yield with a waste rate almost double (15 l m^{-2}) than that permitted. For faba bean, instead, the strong detrimental effect on plant population, suggests a prudential use of OMW not exceeding the minimum dose admitted (8 l m^{-2}), which did not significantly lower the grain yield compared to the control. The highest dose even resulted in the loss of the crop. Durum wheat, notwithstanding the transitory slight phytotoxicity, even reacted effectively to the highest amount of waste evidencing, however, a maximum productive expression to about 10 l m^{-2} rate. Egyptian clover, like forage sorghum, did not suffer particular consequences even with the application of the highest quantities of OMW, but attained the best productive result with a dose (16 l m^{-2}) double than permitted by legislation. The behaviour of barley was comparable to that of the other winter cereal, although the crop reached the highest grain yield with a slightly lower rate of OMW (7 l m^{-2}). Sulla meadow maintained for two years did not experience phytotoxic phenomena performing better than the other tested crops in productive terms, given that it provided the highest seasonal biomass yield with 25 l m^{-2} of OMW. Nevertheless, the response of the last four crops may have been positively influenced by the favourable regimes of rainfall and temperatures following the application of waste performed at an earlier stage of growth (tillering for grain cereals and 3-5 leaf for forage leguminous), which better enabled the plants to confront stress.

At soil level, the overall assessment of the impact determined by the repeated application of the effluent during the long experimental period in presence of the different crops highlighted that there were not relevant consequences on pH and EC, whereas changes occurred for the level of a number of the other analysed features. In general, taking into account the initial uniform pedologic conditions of each the examined layer, a certain final decline in carbonates content and organic matter pool against an increase in the concentration of macronutrients, which was slight for total N and pronounced for assimilable P and K, as well as in the S content was observed. An enhancement was also found for C.E.C. and between the exchangeable cations for Ca in particular, which because of a simultaneous Mg decreasing altered appreciably the equilibrium Ca/Mg. The risk of sodification was lowered compared with the initial soil condition. Among the analysed micronutrient, only B showed a consistent positive variation at the end of the experiment in contrast to a reduction of the others. Considering the final variations between the values of the studied treatments for each soil characteristic in the two layers and taking into account the performance of the crops grown without supplementary fertilizer input, it is possible to infer that the main added value in terms of fertilising effect of the waste is attributable to the macronutrients, especially P an K. The enhancement in comparison to untreated control was generally most marked with the greater doses of OMW with no critical conditions in terms of toxicity towards the crops, excluding faba bean, pollutant risk for groundwater and soil degradation. Consequently, the results clearly demonstrate that even exceeding the amount of the untreated OMW legally permitted within certain limit, does not have unfavourable consequences on growth of the different tested crops or lower their productivity, with the exception of grain legume.

In conclusion, the research provided an insight into a concrete perspective of agronomic recycling of OMW in cropping systems typical of the semi-arid environments of southern Italy even during peak periods of oil mill activity, although the impact on the soil and water table level resulting from the long-term accumulation of potentially polluting compounds require further detailed studies on farming.

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