Use of solid anaerobic digestate and no-tillage practice for restoring the fertility status of two Mediterranean orchard soils with contrasting properties

Giuseppe Badagliacca[a], Beatrix Petrovičovà[a], Shamina Imran Pathan[a,1], Angela Roccotelli[a], Maurizio Romeo[a], Michele Monti[a], Antonio Gelsomino[a,*]

[a] Department of Agricultural Sciences, Mediterranean University of Reggio Calabria, Feo di Vito, 89124 Reggio Calabria, Italy

[1] Present address: Department of Agrifood Production and Environmental Sciences, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy

Giuseppe Badagliacca, ORCID 0000-0002-7688-6210

* Corresponding author at: *Department of Agricultural Sciences*, *Mediterranean University of Reggio Calabria, Feo di Vito, 89124 Reggio Calabria, Italy. Tel.: +39.0965.1694361; fax +39.0965.1694550. E-mail address: agelsomino@unirc.it (A. Gelsomino).* ORCID 0000-0001-5915-7625

Highlights

- In orchard soils aggregate stability declined under conventional tillage, remained unaltered under no-tillage, improved after digestate amendment.
- Following application of solid anaerobic digestate, opposite responses in microbial C-use efficiency and soil N balance were found depending on soil textural and chemical properties.
- No-tillage exerted greater beneficial effects on C-use efficiency and microbial biomass in the coarse than in the fine-textured soil.
- Magnitude and persistence of soil responses were influenced by soil texture and carbonate content.

Abstract

Soil structure degradation, declining soil organic matter and nutrient losses are among major drawbacks of continuous conventional tillage with large-scale environmental consequences including decreasing soil productivity, groundwater contamination and greenhouse gases emissions. This becomes especially true in conventionally-tilled Mediterranean croplands which are also affected by severe climatic conditions. In this study, a one-year field experiment was carried out to investigate the impact of different tillage practices on the soil fertility status in two fruit tree orchards (olive, citrus) with contrasting soil texture (clay, sandy loam), carbonate content (non-calcareous, slightly calcareous) and pH (strongly acid, slightly alkaline), located in Southern Italy. Treatments included in this study were conventional tillage, conventional tillage combined with the incorporation of solid anaerobic digestate, and no-tillage. Changes in the aggregate stability and dynamics of various C and N pools were assessed by monitoring a large set of physical (aggregate stability index), chemical (pH, electrical conductivity, total organic C, total N, nitrate-N, ammonium-N, total soluble N, extractable organic N), biochemical (microbial biomass C and N, basal respiration, potentially mineralizable N) and eco-physiological (microbial and metabolic quotients, mineralization coefficient) soil variables. Results showed that the stability of soil aggregates declined under conventional tillage, remained unaltered under no-tillage, improved after digestate amendment. Moreover, following incorporation of digestate large and long-lasting increase of the organic pool, microbial C-use efficiency and release of soluble C and N forms were observed in the fine-textured soil. Whereas opposite responses were found in the moderately coarse alkaline soil, where incorporation of digestate stimulated C resources depletion, microbial respiration and N losses due to ammonia volatilization with less beneficial effects on soil organic pools. On the other hand, no-tillage prevented soil C and N resources from over-exploitation (as observed in conventionally-tilled soils) with greater beneficial effects on C-use efficiency and microbial biomass found in the coarse than in the fine-textured soil. Our findings suggest that improved management practices such as no-tillage or conventional tillage combined with incorporation of solid anaerobic digestate should specifically deal with soil and climate conditions to became effective for restoring the fertility status in Mediterranean orchard soils.

Keywords

Aggregate stability; *Citrus sinensis* (L.) Osbeck; microbial C-use efficiency; N dynamics; *Olea europaea* L.; soil organic matter; soil texture

1. Introduction

In European semi-arid Mediterranean regions agricultural management with deep tillage and no organic fertilization is a major determinant of accelerated erosion, decline of soil organic matter, loss of nutrients and depletion of soil functions with a consequent reduction of crop yields, and eventually desertification (Zdruli et al., 2010; Lal and Stewart, 2013). As long as the protection of non-renewable soil resources has become a world-wide contemporary task in agriculture, improved management practices have been proposed as urgent measures in Mediterranean agricultural soils to contrast soil degradation while ensuring food security and mitigating greenhouse gases emissions (Holland, 2004; Wezel et al., 2015; Chabert and Sarthou, 2020).

Perennial crops (such as olive, citrus, almond, grapevines, peach, apricot) represent approximately 16% of the agroecosystems in the Mediterranean area with great economic importance (Morugán-Coronado et al., 2020). Some of the most typical characteristics of Mediterranean orchards are the presence of mono-cropping with long tree spacing, mostly rainfed farming, and frequent tillage to avoid the growth of vegetation in the alleys. Since soil remains bare practically all year (Parras-Alcántara et al., 2016), thus, a successful strategy to reduce the negative effects of woody cropping systems on soil is to manage tillage. Replacing conventional practices with reduced or no-tillage practices or providing incorporation of organic amendments have been successfully proposed as improved management systems to overcome losses of soil, nutrients and soil organic matter (Debiase et al., 2016, 2018; Montanaro et al., 2017; Fiore et al., 2018). In recent years, a number of organic by-products from agro-industrial and agro-energy activities have become increasingly available whose use is of great interest for managing the fertility of the soil especially in the Mediterranean area. Among these by-products, appears the digestate that constitutes the end-product of the anaerobic digestion (AD) process of mixed organic wastes for the biological production of the biogas, a gaseous mixture of methane (50-80% v/v) and carbon dioxide used for generating energy and heat (Chynoweth et al., 2001). According to the European Biogas Association, the currently active 17.783 biogas plants contribute to the production of renewable energy with an installed electric capacity of 10.532 MW (EBA, 2018). The production of digestate is actually estimated as large as 20 m^3 yr^{-1} per KW installed (Vilanova Plana and Noche, 2016) with an increasing trend of total amounts because AD plants are expected to increase in the future. Ordinarily, anaerobic digestate is made up of two main fractions according to the dry matter content: a liquid fraction (dry matter 2-8%) and a solid fraction (dry matter 22-30%) (Tambone et al., 2010; Kuusik et al., 2017). The solid fraction is characterized by an alkaline pH, total carbon (C) concentration of about 400 g kg^{-1} with small differences among digestates, total nitrogen (N) content ranging from 15 to 150 g kg^{-1} mostly represented by the ammonium-N form (up to 67%), phosphorus (P) concentration variable from 0.2 to 70 g kg^{-1} and a relatively large potassium (K) content (from 1 to 100 g kg^{-1}) (Teglia et al., 2011; Makádi et al., 2012; Tambone and Adani, 2017; Beggio et al., 2019; Maurer et al., 2019). Nutrient content together with partially-decomposed organic substrates make the solid anaerobic digestate of potential use in agriculture as a substitute of synthetic fertilizers or as a soil conditioner. Nevertheless, large amendment with solid anaerobic digestate can affect soil chemical properties such as the pH (Kataki et al., 2017; Cardelli et al., 2018) and the electrical conductivity (Posmanik et al., 2017). Moreover, the large amount of ammonium-N entering the soil can stimulate the nitrification process thus increasing the nitrate-N pool available to crops or potentially leachable (Alburquerque et al., 2012a; Makádi et al., 2012; Abubaker et al., 2015; Maucieri et al., 2017). Furthermore, digestate supplies soil with partially decomposed organic materials which accumulate or promote microbial respiration with contrasting effects on soil C budget and physical properties (Odlare et al., 2008; Beni et al., 2012; Frøseth et al., 2014). Finally, soil microbial biomass provided contradicting responses: in some cases it increased significantly (García-Sánchez et al., 2015a; Fernández-Bayo et al., 2017; Muscolo et al., 2017), in other cases it showed a small transient increase (Johansen et al., 2013), in others it showed no effect (Makádi et al., 2012). To sum up, benefits from improved soil management practices are not always simple to predict or fully achieve, especially when applied for a short-term period, because their effects can be highly site specific due to soil and climate conditions (Minoshima et al., 2007; Aguilera et al., 2013; Boukhdoud et al., 2016; Badagliacca et al., 2018).

Given these premises, our research aimed to investigate the effects of two improved soil management practices (organic amendment with solid anaerobic digestate and no-tillage) on the fertility status of two Mediterranean orchard soils with contrasting texture, carbonate content and pH. To this aim, a large set of physical (soil aggregate stability index), chemical (pH, electrical conductivity, total organic C, total N, nitrate-N, ammonium-N, total soluble N, extractable organic N), biochemical (microbial biomass C and N, basal respiration, potentially mineralizable N) and eco-physiological (microbial and metabolic quotients, mineralization coefficient) soil variables were monitored following the treatments in an olive and a citrus orchard soil over one-year study period. Results from the organically managed and the no-tilled plots were compared to those from conventionally tilled plots. Three major hypotheses were here tested: 1) amendment with anaerobic digestate contributes soil fertility with a long-lasting release of soluble C and N forms (H1); 2) soil textural properties do greatly affect magnitude and persistence of digestate-induced effects (H2); 3) no-tillage exerts similar effects on soil C and N pools and their dynamics in a way irrelevant to the soil type (H3).

2. Materials and methods

2.1 Solid anaerobic digestate

Solid anaerobic digestate was provided by a local medium-scale biogas producing plant (< 1 MW) operating under mesophilic conditions (T ~40 °C). The biogas plant was supplied with 70% animal manures (cow and poultry), solid wastes from citrus and olive processing plants, pruning materials, maize silage, crop residues (20%), and milk serum (10%). The rated power of the plant is 999 kWh with a hydraulic retention time (HRT) of 60 days in two continuously stirred tank reactors (CSTR) of a total capacity of 7500 m^3 (2500 m^3 tank reactor 1 + 5000 m^3 tank reactor 2). The total volume loaded per day is 120 m^3, the hydraulic retention time (HRT) is 60 days, the minimum guaranteed retention time (MGRT) is 16 h at 40 °C. The resulting digestate was mechanically separated into the aqueous fraction (named liquor), which was discarded, and the solid fraction, which was collected and characterized (Table 1) according to Tambone et al. (2010) and Bonetta et al. (2014) before being used in the present experiment.

2.2 Study sites

The field experiment was established during the 2015/2016 growing season in two orchard sites (an olive and a citrus grove) located within the Calabrian region (Southern Italy) showing contrasting soil texture, carbonate content and pH (Fig. 1).

The olive (*Olea europaea* L. cv. *Carolea*; 70-year old plants with a planting distance of 6 × 6 m) orchard is located in the area nearby Lamezia Terme (Catanzaro, 38˚58’ N, 16˚18’ E, 81 m above the sea level) and is characterized by mild and rainy winters and relatively warm and dry summers. Mean annual rainfall and air temperature are, respectively, 1094 mm and +14.3°C (averages over the 1985-2015 period) (ARPACAL, 2018). Soil thermal and moisture regimes are thermic and udic (first 150 cm), respectively (ARSSA, 2003). The soil is classified as Typic Hapludalf fine, mixed thermic (Soil Survey Staff, 2010) or Cutanic Profondic Luvisol (IUSS Working Group WRB, 2006). The soil is an acid clayey soil (Table 2) and has been kept continuously cultivated with olive trees since mid-50s and since then periodically tilled (till layer 0-20 cm).

The citrus (*Citrus sinensis* (L.) Osbeck cv. *Tarocco*; 30-year old plants with a planting distance of 4 × 4 m) orchard is located near Locri (Reggio Calabria, 38˚13’ N, 16˚14’ E, 12 m above the sea level) in an area with mild rainy winters and arid and warm summers where mean annual rainfall and air temperature are, respectively, 792 mm and +18.3°C (averages over the 1988-2015 period) (ARPACAL, 2018), Soil thermal and moisture regimes are thermic and xeric (first 150 cm), respectively (ARSSA, 2003). The soil is classified as Typic Xerofluvent (Soil Survey Staff, 2010) or Fluvi Calcaric Cambisol (IUSS Working Group WRB, 2006). The soil is a slightly calcareous sandy loam soil (Table 2) and has been cultivated with orange trees for the past 30 years and conventionally tilled to the depth of 20 cm.

2.3 Experimental design and soil treatments

At each site, the experimental set up consisted of field plots (75 m × 18 m each) arranged in a randomized complete block design, with four replications, in order to compare the following three treatments: 1) no-tillage (NT), where weeds were controlled by mechanical mowing and their biomasses was left on soil surface as a residue mulch; 2) conventional tillage (TILL), which consisted of an inter-row harrowing (~20 cm) followed by a slight rolling; 3) digestate incorporation (DIG), which comprised the TILL treatment combined with soil incorporation of solid digestate at a rate of 30 Mg ha^{-1}. This dose, established by considering digestate dosages commonly used in agriculture, is also similar to that used by other authors in C and N mineralization field experiments using organic conditioners (Barra Caracciolo et al., 2015; Fernández-Bayo et al., 2017). According to traditional practices, all field plots were fertilized with an amount of 400 kg ha^{-1} of a 20N-10P_2O_5-10K_2O chemical fertilizer supplying 80 kg N ha^{-1}, 18 kg P ha^{-1} and 34 kg K ha^{-1}.

2.4 Soil sampling

Soil samples were collected 6 days before (T0, early May) and then 2 days (T1, May), 7 weeks (T2, late June), 18 weeks (T3, mid-September) and one year (T4, early May) after the treatments application. Three individual non-rhizosphere soil cores (approx. 200 g each) were surface collected (Ap horizon, 0-20 cm soil layer) from the middle of each of the three inter-row space, so as to minimize any border and plant effect, and then thoroughly mixed to form a unique composite sample. Four composite samples (each from 9 individual inter-row soil cores) were taken per treatment. Twelve composite soil samples were collected (3 treatments × 4 replicates) at each sampling time giving a total number of 60 composite soil samples at the end of the experiment. The same procedure was applied to both experimental sites thus producing, overall, 120 composite soil samples. On return to the laboratory, each sample was split in two aliquots: a representative amount of field moist soil (300 g) was promptly (within 24 h) processed for biochemical analyses; whereas the remaining aliquot (300 g) was air-dried, sieved to pass through a 2-mm sieve, and then stored at room temperature before physical and chemical characterization.

2.5 Soil physical, chemical and biochemical variables

The stability of soil aggregates was determined by measuring the soil aggregate stability index (ISS) on a dry soil using the wet sieving apparatus (Eijkelkamp Agrisearch Equipment, The Netherlands) according to Kemper and Rosenau (1986). Soil chemical properties were determined according to the standard methods recommended by the Soil Science Society of America (Sparks, 1996). Briefly, soil acidity was potentiometrically measured in a 1:2.5 (w/v) soil-to-0.01 M $CaCl_2$ solution mixture (pH_{CaCl2}); electrical conductivity was measured at 25 °C in a 1:2 (w/v) soil-to-water ratio slurry ($EC_{1:2}$ 25°C). Total organic C and N were analyzed by an elemental analyzer LECO CN628 (LECO Corporation, MI, USA). Exchangeable ammonium-N (NH_4^+-N) and soluble nitrate-N (NO_3^-–N) in 2 M KCl soil extracts (1:10, w/v) were determined colorimetrically by the Berthelot reaction and Griess-Ilosvay method, respectively, by using a Flow Injection Analysis System (FIAS 400 PerkinElmer, Inc., CT, USA) equipped with an AS90 Autosampler

(PerkinElmer) and linked to a UV/Vis spectrophotometer Lambda 25 (PerkinElmer). KCl soil extracts were also used to determine the total soluble N (TSN) by using an elemental analyzer TOC-L_{CSH} Shimadzu (Shimadzu Corporation, Tokyo, J) equipped with the TMN-L module for total N determination and an ASI-L Autosampler (Shimadzu). The extractable organic N (EON) was calculated as the difference between the TSN (Shimadzu method) and the sum of NH_4^+-N and NO_3^--N (FIAS method).

The microbial biomass C and N (MBC and MBN) were determined following the chloroform fumigation-extraction (CFE) procedure using a conversion factor of $K_{EC} = 0.45$ and $K_{EN} = 0.54$, respectively (Joergensen et al., 2011). The soil basal respiration (R_{bas}) was determined as described by Öhlinger (1995). The cumulative CO_2-C evolved during a 28-day incubation period (readings after 1, 4, 7, 14, 21 and 28 days of incubation) was assumed as R_{bas}. The potentially mineralizable N (PMN), resulting from net mineralization of the active soil organic N pool during the 28-day incubation period of R_{bas} determination, was estimated as the cumulative soil inorganic-N released after the 28 days of incubation minus the cumulative inorganic soil N at day 0 (Drinkwater et al., 1996). The C and N content in soil extracts for MBC and MBN determination and CO_2 content trapped in the soda solution during the R_{bas} incubation were analyzed by an elemental analyzer TOC-L_{CSH} (Shimadzu). The following derived soil eco-physiological indices (Anderson, 2003; Laudicina et al., 2012) were calculated to assess the impact of improved management practices on soil microbial functioning: the microbial quotient (MBC:C_{org}), the metabolic quotient (qCO_2), the mineralization coefficient ($qM = R_{bas}:C_{org}$).

2.6 Statistics

Soil chemical and biochemical data, reported as mean values ($n = 4$), were expressed on a dry weight (dw) basis (105°C, 24 h). They were first tested for deviation from normality (Kolmogorov-Smirnov test) and homogeneity of within-group variances (Levene’s test). Three-way analysis of variance (ANOVA) (Soil × Time × Management) evidenced a constantly high significant ($P <$

0.001) effect of soil type and its interactions on the variability of all data. Therefore, in order to highlight the effect of time and soil management data from both soils were all considered as replicated measurements and then processed by a two-way ANOVA (Time × Management). Data shown in Figs 2-8 were analyzed by a multiple pairwise comparison of means (Tukey's HSD test at $P < 0.05$). Statistical analysis was performed by using a SAS 9.3 software (SAS Institute, Cary, NC, USA), while all graphs were drawn by using a SigmaPlot v10 software (Systat Software Inc., San Jose, California, USA).

3. Results

3.1 Soil aggregate stability index

Soil ISS was significantly affected by the soil type and its interactions with time and management (three-way ANOVA; Table 3). Moreover, the two-way ANOVA showed a significant effect of soil management and its interaction with time, but not of the sampling time *per se* (Table 3). Despite the soil type, the ISS remained constant in no-tilled plots. Conversely, the amendment with solid anaerobic digestate increased ISS values in both soils, with an immediate, more pronounced (up to 95%) and more extended effect in the olive (from May to September) than in the citrus (from July to September, maximum value 84%) grove soil (Fig. 2). Furthermore, conventional tillage (TILL) determined a reduction of ISS values by 19% and 18% respect to NT in the olive and citrus grove soil, respectively. This effect was still noticeable one month (T2) and four months (T3) after the beginning of the trial. Nevertheless, any significant difference among treatments disappeared at the last sampling time (T4, one year after) (Fig. 2).

3.2 Electrical conductivity and pH

Soil EC and pH were significantly affected by the soil type and its interactions with soil management and sampling time (three-way ANOVA; Table 3). Moreover, the two-way ANOVA revealed that time and soil management significantly influenced EC in either soil (Table 3).

Precisely, no-tillage did not produce any significant variation in EC at both the experimental sites throughout the experimental period. On the contrary, the amendment with solid digestate exerted a pronounced and positive effect on EC in both soils, with a marked initial response (+205% respect to T0) followed by a gradual decline over time in the olive grove soil, and a smaller increase (+62% respect to T0) and a more constant trend in the citrus grove soil (Fig. 3); the highest recorded values were 485 and 396 µS cm^{-1} in the olive and citrus grove soil, respectively. Yet, soil tillage had no effect in the citrus grove soil, whereas in the olive grove soil it raised the EC at the first two sampling times (May and June) by approx. 50% respect to T0 level (Fig. 3). Finally, no significant effect was observed on soil pH (two-way ANOVA, $P > 0.05$; Table 3), which remained practically unaffected in all treatments across the whole experimental period (Fig. 3).

3.3 Total organic carbon and total nitrogen

The three-way ANOVA evidenced that soil type and most of its interactions significantly affected both C_{org} and TN (Table 3). Nevertheless, C_{org} and N contents were somewhat different between the two tested soils: 20.6 *vs* 13.4 g C_{org} kg^{-1} and 1.8 *vs* 1.2 g TN kg^{-1}, respectively, in the olive and in the citrus grove soil (Table 2). On the other side, the two-way ANOVA evidenced that either C_{org} or TN were significantly affected only by soil management (Table 3). Precisely, amendment with solid digestate immediately increased C_{org} and TN in both soils to, respectively, 28.8 g C kg^{-1} and 2.6 g N kg^{-1} in the olive grove and 16.2 g C kg^{-1} and 1.6 g N kg^{-1} in the citrus grove soil (Fig. 4). Interestingly, the two tested soils responded selectively to the application of solid digestate: the increase in total C and N pools showed a significantly more pronounced and longer-lasting effect in the clay than in the sandy loam soil. It is also noteworthy that in the olive orchard soil no-tillage brought about a slight increase of C_{org} and TN (+15%, on average) still appreciable at the end of the experimental period (Fig. 4). In the citrus grove soil no significant difference among treatments was found at the final stage.

3.4 Labile C pools

The three-way ANOVA showed that MBC, albeit statistically affected by Soil × Time and Soil × Management interactions, was not influenced by the soil type ($P > 0.05$; Table 4). Moreover, the two-way ANOVA confirmed that time, soil management and their interaction significantly influenced the variability of MBC (Table 4). The effects of the treatments on the two soils were contrasting, selective and different in magnitude. In particular, in the olive grove soil the DIG treatment markedly stimulated a sudden increase of MBC (from 437 to 770 µg C g^{-1}, at T1), which then gradually declined over time to final 605 µg C g^{-1} (Fig. 5). Conversely, MBC dynamics was less clear in the citrus grove soil where the marked time-dependent fluctuations observed in the DIG treatment were in most cases consistent with those of the TILL treatment; except at the last sampling time where a 1.5-fold increase (equal to 750 µg MBC g^{-1}) was noticed in DIG, whereas final TILL values (497 µg MBC g^{-1}) were same as at the beginning of the trial (Fig. 5). In the olive grove soil tillage (TILL) produced a short-lived MBC increase appreciable only two days after the treatment (T1), while time-dependent fluctuations of MBC values similar in TILL and NT were observed at the remaining sampling times, before reaching final values (range 376 µg MBC g^{-1}) slightly lower than the initial ones. Interestingly, in the citrus orchard soil MBC increased constantly and smoothly in no-tilled plots reaching a final level as high as 653 µg MBC g^{-1} (Fig. 5). To sum up, the following final trend DIG (+15%) > NT (+31%) > TILL was observed (Fig. 5).

Soil type and its interactions with management and sampling time significantly influenced the variability of the microbial quotient data (MBC:C_{org}) (three-way ANOVA; Table 4). On the other side, the two-way ANOVA highlighted that only the sampling time had a significant effect on the variability of MBC:C_{org} data (Table 4). In brief, marked time-dependent fluctuations were found in both soils, even though different responses among treatments could be noticed at later stages (Fig. 5). In particular, in the olive grove soil the MBC:C_{org} ratio followed the trend TILL > DIG > NT; whereas in the citrus grove soil it followed almost the same trend observed for MBC, but with a final increase in the DIG treatment (Fig. 5).

Functional variables such as R_{bas}, qCO_2 and *q*M were significantly affected by the soil type and its interactions with sampling time and soil management (three-way ANOVA; Table 4). The two-way ANOVA showed a significant effect of time, soil management and their interaction on all biochemical variables, with the exception of qCO_2 for which the T × M interaction was not significant ($P > 0.05$; Table 4).

Beside time-dependent fluctuations, no marked variations of R_{bas}, qCO_2 and *q*M were found in no-tilled plots at both sites throughout the experimental period (Fig. 6). However, in the olive orchard final R_{bas} and *q*M values when were lower than the initial ones. Conversely, soil tillage either not (TILL) or combined (DIG) with digestate incorporation stimulated significant changes of R_{bas} and qCO_2, which were different in magnitude as well as in duration depending on the soil type. In details, TILL induced an immediate (T1) but momentary increase of R_{bas}, which then sharply (olive orchard) or slightly (citrus orchard) declined over time to a final value similar to the initial one (Fig. 6). As for the DIG treatment, R_{bas} strongly and immediately (T1) increased in the olive grove soil (1520 µg CO_2-C g^{-1} 28 d^{-1}, +288% respect to pre-treatment) followed by a steadily declining trend to a final value (588 µg CO_2-C g^{-1} 28 d^{-1}) higher than the initial one (+50% respect to pre-treatment); whereas in the citrus grove soil the highest R_{bas} value was reached at T2 (four weeks after the treatment) (981 µg CO_2-C g^{-1} 28 d^{-1}, +140% respect to pre-treatment) and then it steadily decreased to final a value not statistically different among treatments (Fig. 6). Reduced and short-lived changes of qCO_2 were observed in the olive orchard soil, where significant increases were observed two days (T1, DIG) and four weeks (T2, TILL and DIG) after the treatment event (Fig. 6). On the other hand, in the citrus grove soil TILL and DIG treatments brought about a more pronounced increase of qCO_2 (following the trend DIG > TILL) with a lasting effect still appreciable four months (T3) after the beginning of the trial (Fig. 6). No difference of qCO_2 among treatments was observed in both soils at the final sampling. Likewise, R_{bas}, soil tillage stimulated the *q*M with a different trend between the two tested soils: an immediate and transient increase noticeable at T1 followed by a steady decline in the olive grove soil; a more pronounced and longer-lasting increase (noticeable at T1 and T2) in the citrus grove soil (Fig. 6). The addition of solid anaerobic digestate produced an immediate and long-lasting increase of the *q*M still noticeable four months after the beginning of the trial (T4). In both TILL and DIG treatments final *q*M values were the same as the initial ones (Fig. 6).

3.5 Labile N pools

The three-way ANOVA evidenced that labile soil N pools, namely exchangeable NH_4^+-N, NO_3^--N, EON, TSN, MBN and PMN, were significantly affected by all the experimental factors: soil type, sampling time and soil management, as well as by their interactions (Table 5). The two-way ANOVA confirmed that time, soil management and their interaction significantly influenced the variability of the above-mentioned N-pools, with the only exception of the Time × Management interaction on MBN data (Table 5).

Beside noticeable time-dependent fluctuations, the amendment with solid anaerobic digestate raised considerably the exchangeable NH_4^+-N and NO_3^--N content with a major impact observed in the olive than in citrus orchard soil (Fig. 7). In particular, in the olive grove soil the largest NH_4^+-N (25.2 µg N g^{-1}) and NO_3^--N (25.6 µg N g^{-1}) content were recorded one month after the start of trial (at T2); then these values steadily declined to final values (8.8 µg NH_4^+-N g^{-1} and 4.5 µg NO_3^--N g^{-1}), which were higher than the initial ones (Fig. 7). Conversely, in the citrus grove soil the largest NH_4^+-N concentration (8.98 µg N g^{-1}) was found four months after the treatment event (at T3), whereas the highest soil NO_3^--N concentration (30.1 µg N g^{-1}) occurred one month from the beginning of the trial (T2); same as before final levels (6.0 µg NH_4^+-N g^{-1} and 10.8 µg NO_3^--N g^{-1}) were substantially larger than the initial ones (Fig. 7). Beside time-dependent fluctuations, exchangeable NH_4^+-N and NO_3^--N did not significantly vary in conventionally tilled and no-tilled plots and they remained practically unaffected by the treatment (except at T2 in the citrus grove soil) in both soils over the 1-year observation period.

Even though with differences due the soil type, EON readily and temporarily raised following the amendment with solid digestate. In the olive orchard an amount as high as 79.4 µg N g^{-1} was found only at T1 (corresponding to an average +105% compared to both NT and TILL), and then it decreased over time until reaching a final value similar to that observed in NT and TILL (~21 µg N g^{-1}) (Fig. 7). In the citrus grove soil the EON increase was less marked and noticeable within the first month (at T1 and T2), and then no statistically significant differences among soil treatments were found at the following sampling times (Fig. 7). As for the inorganic-N pool, EON showed only time-dependent fluctuations in conventionally tilled and no-tilled plots at both sites throughout the experimental period. No difference of EON among treatments was observed in both soils at the final sampling (Fig. 7).

The amendment with solid digestate resulted in a marked, immediate and long-lasting increase of TSN in both soils, with a general trend in line with what already observed for NH_4^+-N and NO_3^--N (Fig. 7). Briefly, in the clay soil (olive site) after a sudden and marked increase at T1 (up to 115.1 µg N g^{-1} equal to +145% respect to the other treatments), TSN steadily declined (to 85.5 and 65.5 µg N g^{-1} at T2 and T3, respectively), until reaching a final value similar to the initial one and not significantly different with the other treatments (NT and TILL). Likewise, in the sandy loam soil (citrus site), digestate-induced TSN increases were less pronounced but longer-lasting, being still visible at the end of the experiment (34.4 µg N g^{-1}, +29% on average) (Fig. 7). In conventionally tilled and no-tilled plots TSN remained substantially unaffected at both sites and, despite concurrent time-dependent variations, final TSN values were not as different from the initial ones (Fig. 7).

In general, MBN dynamics were consistent with those already observed for MBC, showing selective responses in relation to the soil type. In brief, amendment with solid anaerobic digestate stimulated a marked, immediate and long-lasting increase of MBN in the olive grove soil; whereas in the citrus grove soil MBN responses were only noticed at the final stage (Fig. 8). Thus, final MBN values in DIG were higher than at the beginning of the trial at both experimental sites. As for the olive grove soil, time-dependent variations were observed in TILL and NT treatments with final

MBN values same as at the beginning of the trial (Fig. 8). On the contrary, in the citrus grove soil MBN values in no-tilled plots showed a slight but constant increase more evident at the end of the trial, thus confirming the already seen trend for MBC at the final stage DIG (+38%) > NT (+90%) > TILL (Fig. 8).

As well as for TSN, a similar trend in DIG was observed in the PMN which showed higher increases in the clay soil (olive site) and longer-lasting in the sandy loam soil (citrus site). Once again, NT and TILL showed a similarity between initial and final values (Fig. 8).

4. Discussion

It is known that any variation of soil physical and chemical conditions exerts a considerable influence on soil microbial biomass growth, activity and community composition, on nutrient dynamics, and hence on the soil fertility status. This is particularly true for any tillage or soil management event on herbaceous (Jackson et al., 2003; Conant et al., 2007; Zuber and Villamil, 2016) or woody crops (Sofo et al., 2014; Montanaro et al., 2017; Morugán-Coronado et al., 2020) as well as soil incorporation of anaerobic digestate (Alburquerque et al., 2012a, 2012b; Abubaker et al., 2015; Šimon et al., 2015), which impact on soil physical, chemical and biological properties. In soils, physical, chemical and biological changes are interrelated. In a parallel paper in preparation the authors are to show compositional changes in the phylogenetic structure of both total and active soil bacterial communities as induced by the same management treatments in the two orchard soils. Therefore, reporting on changes in soil aggregate stability, as well as in C and N pools and their dynamics was the main aim of the present work.

4.1 Physical and chemical responses

Physical properties were clearly affected in digestate-amended soils as evidenced by the ISS increase found at both sites. This finding is in line with what reported by Beni et al. (2012) and Frøseth et al. (2014) and could be attributed either to the direct binding action of organic polymers from decaying substrates of the digestate (Voelkner et al., 2015) or indirectly to the sticky network of the digestate-stimulated growth of fungal hyphae that controls soil aggregate formation and stability (Andrade et al., 1998; Alburquerque et al., 2012a). Abundance of soil mineral colloids (which protect native soil organic matter from decomposition) together with acidic conditions (conducive to fungal growth) can explain the marked and long-lasting ISS response observed in the olive orchard soil. This finding confirms the key role of soil texture, especially high clay content, in enhancing any positive physical action due to the addition of solid digestate. In line with literature review (Holland, 2004; Plaza-Bonilla et al., 2013), evidence of beneficial effects of no-tillage on maintaining soil structural properties, whatever the soil type, were here further confirmed. On the other side, declining ISS values observed in conventionally tilled plots - especially in the citrus orchard soil where a lower clay content occurring together with drier and warmer climatic conditions - warn farmers about soil degradation risks due to repeated mechanical events. Taken together these findings make the recommendation to adopt proper management practices to maintain (through no-tillage) or improve (through organic amendment) soil aggregate stability, especially in highly vulnerable Mediterranean cropland areas.

We observed that incorporation of a salt-rich substrate such as solid anaerobic digestate increased soil EC in both tested soils. Not unexpectedly, this finding is in agreement with what reported by several authors (García-Sánchez et al., 2015a, 2015b; Gómez-Brandón et al., 2016; Posmanik et al., 2017). This means that the risk of increasing soil salinity due to repeated applications of large amounts of digestate cannot be ignored, especially when soil EC values are next to exceed the threshold level of 2 dS m^{-1}, which negatively interfere with plant growth and reduce crop yield (Gómez-Brandón et al., 2016, Kataki et al., 2017). However, this was not the case since measured EC values were always well below that critical level. Yet, it cannot be ignored that final EC values equal to +94.0 and +72.8 µS cm^{-1} were found in digestate-treated soils at both sites (olive and citrus orchard, respectively) in spite of their different leaching potential. Therefore, we can speculate that the critical EC threshold of secondary salinization would be reached after 15 years in the clay soil and 20 years in the sandy loam soil, provided leaching potential remains constant and the same amount of solid digestate is annually applied. On the other side, addition of digestate left the soil pH practically unaffected, thus confirming that the high clay content and the calcium carbonate system occurring in the olive and the citrus orchard soil, respectively, as a result of the underlying soil formation processes, provide a strong buffering capacity and any significant variation of soil acidity can only arise from very large additions repeated over the very long term (Odlare et al., 2008; Makádi et al., 2012).

Even though the majority of authors reported an increased C_{org} content in digestate-treated soils (Rigby and Smith, 2013; García-Sánchez et al., 2015a; Muscolo et al., 2017; Cardelli et al., 2018), contrasting results were also observed (Alburquerque et al., 2012a; Gómez-Brandón et al., 2016). In fact, the newly added organic matter could have stimulated the microbial activity thus resulting in an increased breakdown of some of the more protected soil organic matter (priming effect), an extra release of soluble C and N forms and eventually depletion of soil nutrient resources (Eickenscheidt et al., 2014; Insam et al., 2015). Since no significant reduction of C_{org} was observed one year after the addition of solid digestate at both sites, we suppose that no priming effect was acting in our soils, or, if any, it remained negligible. On the contrary, larger and longer-lasting organic-C increases were observed in the clayey than in the sandy loam soil, suggesting that fine-textured particles could have promoted the physical protection of the added organic materials, in line with Lützow et al. (2006) and Six and Paustian (2014). Once again, the link between digestate characteristics and soil properties is decisive to whether incorporation of solid digestate can contribute to soil C sequestration or represent a risk for groundwater resources. Likewise, it was observed that the effect of digestate amendment on total soil N was similar to that on C_{org}, in accordance with Hupfauf et al. (2016).

Finally, it is known that avoiding mechanical disturbance leads to low aeration, increased aggregate stability and consequently to a reduced mineralization of the native organic pool

(Holland, 2004). This was clearly evident in the clay olive orchard soil where no-tillage provided beneficial effects on the total organic pool and structural stability.

4.2 Labile C and N pools

MBC, MBN and R_{bas} increases were not unexpected since there is a widely held view that addition of a partially degraded, nutrient-rich end-product such as solid anaerobic digestate has the potential to promote soil microbial growth (Alburquerque et al., 2012a; Barra Caracciolo et al., 2015; Muscolo et al., 2017; Cardelli et al., 2018) and activity (Odlare et al., 2008; Möller, 2015; Hupfauf et al., 2016; Cucina et al., 2018). However, it must be noted that MBC and MBN dynamics were selectively affected by site-specific environmental factors such as climate conditions – namely air temperature and rainfall regimes - and soil type. In fact, soil microbial biomass was reduced during the dry, hot summer period at both sites, thus confirming the key role of soil temperature and moisture in driving seasonal changes in soil (Feng and Simpson, 2009). Moreover, contrasting microbial responses to digestate amendment can be also due to the close interplay between soil microorganisms and their abiotic environment - which is somewhat diverse between the two sites. The marked, immediate and long-lasting MBC (and MBN) rise found in the clayey soil following digestate amendment can be ascribed to either the addition of easily degradable organic compounds or to the increased availability of ecological niches due to tillage-induced breakdown of soil aggregates, as also postulated by Álvaro-Fuentes et al. (2008), Morell et al. (2010) and Zheng et al. (2018). Moreover, since higher but decreasing levels of metabolic activity (followed as qCO_2 and qM) were also recorded, it is plausible to hypothesize that the extra release of soluble C and N forms in digestate-treated soils, as well as the major access to spatial patterns and native stabilized organic matter in conventionally tilled plots, could have altered considerably the patterns of functional microbial activity. In other words, changes in spatial conditions and easier access to nutritional resources caused by tillage combined with the organic amendment could have resulted in an increased microbial C-use efficiency in the clay-rich soil. On the contrary, the same treatment offered a contrasting functional picture in the sandy loam soil. That is, neither did the microbial biomass increase nor the eco-physiological indicators declined. This finding suggests that the major C pool entering the soil was less efficiently used in this soil type, thus leading to the conclusion that the two orchard soils showed a clear difference in their microbial C-use efficiency. Yet, the assumption of some authors (Manzoni et al., 2008; Martin et al., 2015) that microbial C-use efficiency declines under prolonged shortage of available soil N does not find here any convincing experimental support, since large soluble N pools (see for instance inorganic-N, TSN) were readily available soon after digestate addition. Instead, a larger view which considers C pool dynamics also in tilled and no-tilled plots suggests that in the sandy loam soil the tillage event rather than the organic amendment *per se* was mainly responsible for a major stressing condition to native microbial community. In fact, similar trends of MBC and MBC:C_{org} were retrieved in both tilled treatments (either with or without organic amendment); however, the significant microbial biomass increase found only after a reasonable period of time in the digestate-treated soil is probably linked to the beneficial contribution of the organic amendment. To sum up, amendment with solid anaerobic digestate determined long-lasting effects on soil C pools and functional properties as hypothesized (H1). However, soil textural and chemical properties are key to determine whether conventional tillage combined with digestate amendment would benefit C pools and microbial C-use efficiency (as in acid clay soil); or, on the contrary, would stimulate C resources depletion (as in the alkaline sandy loam soil) (also H2 was confirmed). Noteworthy, no-tillage prevented the over-exploitation of soil C resources and this beneficial effect was more evident in the fine-textured soil where a slight increase of the total C pool as well as a higher microbial C-use efficiency was observed (H3 was not confirmed).

Even though solid digestate addition suddenly increased the inorganic-N pools – a finding in line with what previously reported (Jones et al., 2007; Alburquerque et al., 2012a; Eickenscheidt et al., 2014; Martin et al., 2015; Cucina et al., 2018) - marked differences were also noted depending on the soil type. Firstly, trends of soil NH_4^+-N and NO_3^--N, as well as their relative amount, varied considerably between the two soil types and a clear predominance of NO_3^--N over NH_4^+-N in the citrus grove soil was found. Few salient facts are worth noting to explain this finding. A pH exceeding 7.0 together with warmer and drier conditions could have promoted large ammonia volatilization in the sandy loam (citrus) soil. Whereas it is likely that ammonium adsorption and fixation were the prevailing processes in the acid olive orchard soil, which is also characterized by reactive clay surfaces and thus high CEC (51.9 $cmol_+$ kg^{-1}, Table 2). As long as ammonia volatilization occurred, gaseous NH_3-N could have affected negatively the soil microbiota in the citrus grove soil, thus decreasing MBC and MBN levels. Needless to say, the nitrification process is favored by the availability of exchangeable Ca^{2+} and Mg^{2+} ions, mesophilic conditions and good soil aeration, as found in the citrus orchard soil. Whereas it is constrained in soil high in clay content and low in pH (as in the olive orchard). To sum up, contrasting soil physico-chemical properties and differing nitrification rates can help explain the different dynamics of NH_4^+-N, NO_3^--N and TSN observed in the two digestate-treated soils. Yet, slight differences in NO_3^--N and TSN patterns found between TILL and NT treatments in the citrus grove soil, but not in the olive orchard, are likely due to tillage-induced faster mineralization rates, as also confirmed by raised R_{bas} and qCO_2 observed in this soil type.

Incorporation of solid anaerobic digestate into the soil is able to release a significant amount of easily mineralizable organic-N containing substrates (Tambone et al., 2010; Galvez et al., 2012), that can undergo a more or less rapid mineralization depending on their C/N ratio. Since the digestate here used had a C/N ratio as low as 24, the overall result is an increased release of mineral N forms and an altered N pools dynamics, which appear different according to the soil type (thus also confirming H1 and H2). Indeed, in the olive grove soil digestate amendment promoted either the microbial activity (that is C-substrate mineralization) with consequent release of inorganic-N forms or the microbial growth (that is N-immobilization), the latter being responsible for depleting labile C and N pools and delaying the nitrification (in accordance with Alburquerque et al., 2012a and Johansen et al., 2013). In addition, Ros et al. (2009, 2011) noted that clay surfaces can exert a protective action towards EON. This can explain the PMN pattern observed in the olive grove soil. On the contrary, microbial immobilization can be discounted in the citrus grove soil, assuming that mineralization of soluble N-containing organic substrates was the major process and promoted faster N turnover rates. However, we hypothesize that N loss due to ammonia volatilization favored by the alkaline condition might have prevented the soluble N balance from increasing significantly. Finally, besides a slight tillage-induced release of EON noted in the clay soil, in most but not all cases (except MBN) no difference was found in soluble and functional N pool between NT and TILL treatments whatever the soil type (H3 could not be fully confirmed).

5. Conclusions

Maintenance and restoration of soil organic pools and related functional properties represent a major challenge facing modern agriculture especially in conventionally-managed Mediterranean cropland areas where climatic conditions exacerbate the loss of soil, nutrients and organic resources (often below the critical threshold of 2% C_{org}), thus severely threatening the fertility of soils. Within this context, agricultural reuse of biodegradable wastes such as by-products of agro-energy activities is strongly recommended as a farming strategy to restore declining organic resources. However, although amendment with solid anaerobic digestate is of great potential as a soil conditioner, its use should be carefully evaluated in relation to soil and management conditions. Findings of our study carried out in two orchard soils (olive and citrus grove) representative of Mediterranean perennial crops showed that conventional tillage combined with incorporation of solid anaerobic digestate into a fine-textured soil was capable of raising the organic pool with a related beneficial effect on soil structure, microbial C-use efficiency and long-lasting release of soluble C and N forms. On the other side, in the moderately coarse alkaline soil the same treatment stimulated soil C-resources depletion, microbial respiration and N losses due to ammonia volatilization and nitrate leaching. Additionally, no-tillage acted in a way that prevented soil C and N resources from over-exploitation (as observed in conventionally tilled soils) with a greater beneficial effects on microbial C-use efficiency and microbial biomass in the moderately coarse than in the fine-textured soil. To sum up, although our findings showed potential benefits of application of solid anaerobic digestate and no-tillage practice for the restoring the fertility status of orchard soils under Mediterranean climate, strong interactions between magnitude and persistence of these benefits and soil properties were also observed, suggesting that site specific conditions should be duly considered when applying improved management systems.

Acknowledgements

We thank Antonino Zumbo, Tommaso Gullì and Luigi Martire for technical advice and support. This work was supported by the Italian Ministry of Education, University and Research (MIUR) within the frame of the action PON Research and Competitiveness 2007/2013 with the two projects entitled PON03PE_00090_2 (Modelli sostenibili e nuove tecnologie per la valorizzazione delle olive e dell’olio extravergine di oliva prodotto in Calabria) and PON03PE_00090_3 (Modelli sostenibili e nuove tecnologie per la valorizzazione delle filiere vegetali mediterranee).

References

Abubaker. J., Risberg, K., Jönsson, E., Dahlin, A.S., Cederlund, H., Pell, M., 2015. Short-term effects of biogas digestates and pig slurry application on soil microbial activity. Appl. Environ. Soil. Sci. 658542. https://doi.org/10.1155/2015/658542.

Aguilera, E., Lassaletta, L., Gattinger, A., Gimeno, B.S., 2013. Managing soil carbon for climate change mitigation and adaptation in Mediterranean cropping systems. A meta-analysis. Agric. Ecosyst. Environ. 168, 25–36. https://doi.org/10.1016/j.agee.2013.02.003.

Alburquerque, J.A., de la Fuente, C., Bernal, M.P., 2012a. Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. Agric. Ecosyst. Environ. 160, 15–22. https://doi.org/10.1016/j.agee.2011.03.007.

Alburquerque, J.A., de la Fuente, C., Campoy, M., Carrasco, L., Nájera, I., Baixauli, C., Caravaca, F., Roldán, A., Cegarra, J., Bernal, M.P., 2012b. Agricultural use of digestate for horticultural crop production and improvement of soil properties. Eur. J. Agron. 43, 119–128. https://doi.org/10.1016/j.eja.2012.06.001.

Álvaro-Fuentes, J., López, M.V., Cantero-Martinez, C., Arrúe, J.L., 2008. Tillage effects on soil organic carbon fractions in mediterranean dryland agroecosystems. Soil Sci. Soc. Am. J. 72, 541–547. https://doi.org/10.2136/sssaj2007.0164.

Anderson, T.H., 2003. Microbial eco-physiological indicators to assess soil quality. Agric. Ecosyst.

Environ. 98, 285–293. https://doi.org/10.1016/S0167-8809(03)00088-4.

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1998. Soil aggregation status and rhizobacteria in the mycorrhizosphere. Plant Soil 202, 89–96. https://doi.org/10.1023/A:1004301423150.

ARPACAL (Agenzia Regionale per la Protezione dell'Ambiente della Calabria), 2018. Banca dati storici. http://www.cfd.calabria.it (accessed 19/02/2020).

ARSSA (Agenzia Regionale per i Servizi di Sviluppo Agricolo), 2003. I suoli della Calabria - Carta dei suoli in scala 1:250.000. Monografia divulgativa. Rubbettino Industrie Grafiche, CZ.

Badagliacca, G., Benítez, E., Amato, G., Badalucco, L., Giambalvo, D., Laudicina, V.A., Ruisi, P., 2018. Long-term effects of contrasting tillage on soil organic carbon, nitrous oxide and ammonia emissions in a Mediterranean Vertisol under different crop sequences. Sci. Total Environ. 619-620, 18-27. https://doi.org/10.1016/j.scitotenv.2017.11.116.

Barra Caracciolo, A., Bustamante, M.A., Nogues, I., Di Lenola, M., Luprano, M.L., Grenni, P., 2015. Changes in microbial community structure and functioning of a semiarid soil due to the use of anaerobic digestate derived composts and rosemary plants. Geoderma 245-246, 89-97. doi: https://doi.org/10.1016/J.GEODERMA.2015.01.021.

Beggio, G., Schievano, A., Bonato, T., Hennebert, P., Pivato, A., 2019. Statistical analysis for the quality assessment of digestates from separately collected organic fraction of municipal solid waste (OFMSW) and agro-industrial feedstock. Should input feedstock to anaerobic digestion determine the legal status of digestate? Waste Manag. 87, 546-558. https://doi.org/10.1016/j.wasman.2019.02.040.

Beni, C., Servadio, P., Marconi, S., Neri, U., Aromolo, R., Diana, G., 2012. Anaerobic digestate administration: effect on soil physical and mechanical behavior. Commun. Soil. Sci. Plant. Anal. 43, 821-834. https://doi.org/10.1080/00103624.2012.648359.

Bonetta, S., Ferretti, E., Fezia, G., Gilli, G., Carraro, E., 2014. Agricultural reuse of the digestate from anaerobic co-digestion of organic waste: microbiological contamination, metal hazards and fertilizing performance. Water Air Soil Pollut. 225, 2046. https://doi.org/10.1007/s11270-014-2046-2.

Boukhdoud, N., Gros, R., Darwish, T., Farnet da Silva, A.M., 2016. Effect of agricultural practices and coastal constraints on soil microbial functional properties in Mediterranean olive orchards. Eur. J. Soil Sci. 67(4), 470-477. https://doi.org/10.1111/ejss.12347.

Cardelli, R., Giussani, G., Marchini, F., Saviozzi, A., 2018. Short-term effects on soil of biogas digestate, biochar and their combinations. Soil Res., 56, 623-631. https://doi.org/10.1071/SR18017.

Chabert, A., Sarthou, J.P., 2020. Conservation agriculture as a promising trade-off between conventional and organic agriculture in bundling ecosystem services. Agric. Ecosyst. Environ. 292, 106815. https://doi.org/10.1016/j.agee.2019.106815.

Chynoweth, D.P., Owens, J.M., Legrand, R., 2001. Renewable methane from anaerobic digestion of biomass. Renewable Energy. 22(1-3), 1-8. https://doi.org/10.1016/S0960-1481(00)00019-7

Conant, R.T., Easter, M., Paustian, K., Swan, E., Williams, S., 2007. Impact of periodic tillage on C stocks: a synthesis. Soil Till. Res. 95, 1-10. https://doi.org/10.1016/j.still.2006.12.006.

Cucina, M., Tacconi, C., Ricci, A., Pezzolla, D., Sordi, S., Zadra, C., Gigliotti, G., 2018. Evaluation of benefits and risks associated with the agricultural use of organic wastes of pharmaceutical origin. Sci. Total Environ. 613-614, 773-782. https://doi.org/10.1016/j.scitotenv.2017.09.154.

Debiase, G., Montemurro, F., Fiore, A., Rotolo, C., Farrag, K., Miccolis, A., Brunetti, G., 2016. Organic amendment and minimum tillage in winter wheat grown in Mediterranean conditions: Effects on yield performance, soil fertility and environmental impact. Eur. J. Agron. 75, 149-157. https://doi.org/10.1016/j.eja.2015.12.009.

Debiase, G., Traversa, A., Montemurro, F., Mastrangelo, M., Fiore, A., Ventrella, G., Brunetti, G., 2018. Minimum tillage and organic fertilization for the sustainable management of *Brassica carinata* A. (Braun) in the Mediterranean environment. Environ. Sci. Pollut. Res. 25, 33556-33565. https://doi.org/10.1007/s11356-018-3244-z.

Drinkwater, L.E., Cambardella, C.A., Reeder, J.D., Rice, C.W., 1996. Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen, in: Doran, J.W., Jones, A.J. (Eds.), Methods for Assessing Soil Quality. Soil Science Society of America Special Publication No. 49, Madison, pp. 217-229.

EBA, 2018. Statistical Report of the European Biogas Association 2018, European Biogas association, Brussels.

Eickenscheidt, T., Freibauer, A., Heinichen, J., Augustin, J., Drösler, M., 2014. Short-term effects of biogas digestate and cattle slurry application on greenhouse gas emissions affected by N availability from grasslands on drained fen peatlands and associated organic soils. Biogeosciences 11, 6187-6207. https://doi.org/10.5194/bg-11-6187-2014.

Feng, X., Simpson, M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil. Biol. Biochem. 41, 804-812. https://doi.org/10.1016/J.SOILBIO.2009.01.020.

Fernández-Bayo, J.D., Achmon, Y., Harrold, D.R., McCurry, D.G., Hernandez, K., Dahlquist-Willard, R.M., Stapleton, J.J., VanderGheynst, J.S., Simmons, C.W., 2017. Assessment of two solid anaerobic digestate soil amendments for effects on soil quality and biosolarization efficacy. J. Agric. Food Chem. 65, 3434-3442. https://doi.org/10.1021/acs.jafc.6b04816.

Fiore, A., Lardo, E., Montanaro, G., Laterza, D., Loiudice, C., Berloco, T., Dichio, B., Xiloyannis, C., 2018. Mitigation of global warming impact of fresh fruit production through climate smart management. J. Clean. Prod. 172, 3634-3643. https://doi.org/10.1016/j.jclepro.2017.08.062.

Frøseth, R.B., Bakken, A.K., Bleken, M.A., Riley, H., Pommeresche, R., Thorup-Kristensen, K., Hansen, S., 2014. Effects of green manure herbage management and its digestate from biogas production on barley yield, N recovery, soil structure and earthworm populations. Eur. J. Agron. 52, 90-102. https://doi.org/10.1016/j.eja.2013.10.006.

Galvez, A., Sinicco, T., Cayuela, M.L., Mingorance, M.D., Fornasier, F., Mondini, C., 2012. Short term effects of bioenergy by-products on soil C and N dynamics, nutrient availability and biochemical properties. Agric. Ecosyst. Environ. 160, 3-14. https://doi.org/10.1016/j.agee.2011.06.015.

García-Sánchez, M., Garcia-Romera, I., Cajthaml, T., Tlustoš, P., Száková, J., 2015a. Changes in soil microbial community functionality and structure in a metal-polluted site: The effect of digestate and fly ash applications. J. Environ. Manage. 162, 63-73. https://doi.org/10.1016/j.jenvman.2015.07.042.

García-Sánchez, M., Siles, J.A., Cajthaml, T., García-Romera, I., Tlustoš, P., Száková, J., 2015b. Effect of digestate and fly ash applications on soil functional properties and microbial communities. Eur. J. Soil. Biol. 71, 1-12. https://doi.org/10.1016/j.ejsobi.2015.08.004.

Gómez-Brandón, M., Juárez, M.F.D., Zangerle, M., Insam, H., 2016. Effects of digestate on soil chemical and microbiological properties: A comparative study with compost and vermicompost. J. Hazard Mater. 302, 267-274. https://doi.org/10.1016/j.jhazmat.2015.09.067.

Holland, J.M., 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agric. Ecosys. Environ. 103, 1-25. https://doi.org/10.1016/j.agee.2003.12.018.

Hupfauf, S., Bachmann, S., Fernández-Delgado Juárez, M., Insam, H., Eichler-Löbermann, B., 2016. Biogas digestates affect crop P uptake and soil microbial community composition. Sci. Total Environ. 542, 1144-1154. https://doi.org/10.1016/j.scitotenv.2015.09.025.

Insam, H., Gómez-Brandón, M., Ascher, J., 2015. Manure-based biogas fermentation residues - Friend or foe of soil fertility? Soil Biol. Biochem. 84, 1-14. https://doi.org/10.1016/j.soilbio.2015.02.006.

IUSS Working Group WRB, 2006. World Reference Base for Soil Resources. World Soil Resources Reports No. 103, second ed. FAO, Rome.

Jackson, L.E., Calderon, F.J., Steenwerth, K.L., Scow, K.M., Rolston, D.E., 2003. Responses of soil microbial processes and community structure to tillage events and implications for soil quality. Geoderma 114, 305-317. https://doi.org/10.1016/S0016-7061(03)00046-6.

Joergensen, R.G., Wu, J., Brookes, P.C., 2011. Measuring soil microbial biomass using an automated procedure. Soil Biol. Biochem. 43, 873-876. https://doi.org/10.1016/J.SOILBIO.2010.09.024.

Johansen, A., Carter, M.S., Jensen, E.S., Hauggard-Nielsen, H., Ambus, P., 2013. Effects of digestate from anaerobically digested cattle slurry and plant materials on soil microbial community and emission of CO_2 and N_2O. Appl. Soil Ecol. 63, 36-44. https://doi.org/10.1016/j.bbagen.2014.04.007.

Jones, S.K., Rees, R.M., Skiba, U.M., Ball, B.C., 2007. Influence of organic and mineral N fertiliser on N_2O fluxes from a temperate grassland. Agric. Ecosyst. Environ. 121, 74-83. https://doi.org/10.1016/J.AGEE.2006.12.006.

Kataki, S., Hazarika, S., Baruah, D.C., 2017. Investigation on by-products of bioenergy systems (anaerobic digestion and gasification) as potential crop nutrient using FTIR, XRD, SEM analysis and phytotoxicity test. J. Environ. Manage. 196, 201–216. https://doi.org/10.1016/j.jenvman.2017.02.058.

Kemper, W.D., Rosenau, R.C., 1986. Aggregate stability and size distribution, in: Klute, A. (Ed.), Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods. American Society of Agronomy and Soil Science Society of America, Madison, pp. 425-442.

Kuusik, A., Pachel, K., Kuusik, A., Loigu, E., 2017. Possible agricultural use of digestate. Proc. Est. Acad. Sci. 66, 64-74. https://doi.org/10.3176/proc.2017.1.10.

Lal, R., Stewart, B.A., 2013. Principles of Sustainable Soil Management in Agroecosystems. A series in Advances in Soil Science. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Laudicina, V.A., Barbera, V., Gristina, L., Badalucco, L., 2012. Management practices to preserve soil organic matter in semiarid mediterranean environment, in: Bjorklund, P.A., Mello, F.V. (Eds.), Soil Organic Matter: Ecology, Environmental Impact and Management. Nova Science Publishers, New York, pp. 39–61.

Lützow, M., Kogel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. Eur. J. Soil Sci. 57, 426–445. https://doi.org/10.1111/j.1365-2389.2006.00809.x

Makádi, M., Tomócsik, A., Orosz, V., 2012. Digestate: a new nutrient source – Review, in: Kumar, S. (Ed.), Biogas. InTechOpen, Rijeka, pp. 295–310.

Manzoni, S., Jackson, R.B., Trofymow, J.A., Porporato, A., 2008. The global stoichiometry of litter nitrogen mineralization. Science 321, 684-686. https://doi.org/10.1126/science.1159792.

Martin, S.L., Clarke, M.L., Othman, M., Ramsden, S.J., West, H.M., 2015. Biochar-mediated reductions in greenhouse gas emissions from soil amended with anaerobic digestates. Biomass and Bioenergy 79, 39–49. https://doi.org/10.1016/j.biombioe.2015.04.030.

Maucieri, C., Nicoletto, C., Caruso, C., Sambo, P., Borin, M., 2017. Effects of digestate solid fraction fertilisation on yield and soil carbon dioxide emission in a horticulture succession. Ital. J. Agron. https://doi.org/10.4081/ija.2017.800.

Maurer, C., Seiler-Petzold, J., Schulz, R., Müller, J., 2019. Short-term nitrogen uptake of barley from differently processed biogas digestate in pot experiments. Energies 12, 696. https://doi.org/10.3390/en12040696.

Minoshima, H., Jackson, L.E., Cavagnaro, T.R., Sánchez-Moreno, S., Ferris, H., Temple, S.R., Goyal, S., Mitchell, J.P., 2007. Soil food webs and carbon dynamics in response to conservation tillage in California. Soil Sci. Soc. Am. J. 71, 952-963. https://doi.org/10.2136/sssaj2006.0174.

Möller, K., 2015. Effects of anaerobic digestion on soil carbon and nitrogen turnover, N emissions, and soil biological activity. A review. Agron. Sustain. Dev. 35, 1021-1041. https://doi.org/10.1007/s13593-015-0284-3.

Montanaro, G., Xiloyannis, C., Nuzzio, V., Dichio, B., 2017. Orchard management, soil organic carbon and ecosystem services in Mediterranean fruit tree crops. Sci. Hortic. 217, 92-101. http://dx.doi.org/10.1016/j.scienta.2017.01.012.

Morell, F.J., Álvaro-Fuentes, J., Lampurlanés, J., Cantero-Martínez, C., 2010. Soil CO_2 fluxes following tillage and rainfall events in a semiarid Mediterranean agroecosystem: Effects of tillage systems and nitrogen fertilization. Agric. Ecosyst. Environ. 139, 167-173. https://doi.org/10.1016/J.AGEE.2010.07.015.

Morugán-Coronado, A., Linares, C., Gómez-López, M.D., Faz, Á., Zornoza, R., 2020. The impact of intercropping, tillage and fertilizer type on soil and crop yield in fruit orchards under Mediterranean conditions: A meta-analysis of field studies. Agric. Syst. 178, 102736. https://doi.org/10.1016/j.agsy.2019.102736.

Muscolo, A., Settineri, G., Papalia, T., Attinà, E., Basile, C., Panuccio, M.R., 2017. Anaerobic co-digestion of recalcitrant agricultural wastes: Characterizing of biochemical parameters of digestate and its impacts on soil ecosystem. Sci. Total Environ. 586, 746-752. https://doi.org/10.1016/j.scitotenv.2017.02.051.

Odlare, M., Pell, M., Svensson, K., 2008. Changes in soil chemical and microbiological properties during 4 years of application of various organic residues. Waste Manage. 28, 1246-1253. https://doi.org/10.1016/J.WASMAN.2007.06.005.

Öhlinger, R., 1995. Soil respiration by titration, in: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), Methods in Soil Biology. Springer, Berlin, pp. 95-98.

Parras-Alcántara, L., Lozano-García, B., Keesstra, S., Cerdà, A., Brevik, E.C., 2016. Longterm effects of soil management on ecosystem services and soil loss estimation in olive grove top soils. Sci. Total Environ. 571, 498–506. https://doi.org/10.1016/J.SCITOTENV.2016.07.016.

Plaza-Bonilla, D., Cantero-Martínez, C., Viñas, P., Álvaro-Fuentes, J., 2013. Soil aggregation and organic carbon protection in a no-tillage chronosequence under Mediterranean conditions. Geoderma 193, 76-82. https://doi.org/10.1016/j.geoderma.2012.10.022.

Posmanik, R., Nejidat, A., Dahan, O., Gross, A., 2017. Seasonal and soil-type dependent emissions of nitrous oxide from irrigated desert soils amended with digested poultry manures. Sci. Total Environ. 593-594, 91-98. https://doi.org/10.1016/j.scitotenv.2017.03.115.

Rigby, H., Smith, S.R., 2013. Nitrogen availability and indirect measurements of greenhouse gas emissions from aerobic and anaerobic biowaste digestates applied to agricultural soils. Waste Manage. 33, 2641-2652. https://doi.org/10.1016/j.wasman.2013.08.005.

Ros, G.H., Hoffland, E., van Kessel, C., Temminghoff, E.J.M., 2009. Extractable and dissolved soil organic nitrogen - A quantitative assessment. Soil Biol. Biochem. 41, 1029-1039. https://doi.org/10.1016/j.soilbio.2009.01.011.

Ros, G.H., Hanegraaf, M.C., Hoffland, E., van Riemsdijk, W.H., 2011. Predicting soil N mineralization: Relevance of organic matter fractions and soil properties. Soil Biol. Biochem. 43, 1714-1722. https://doi.org/10.1016/j.soilbio.2011.04.017.

Šimon, T., Kunzová, E., Friedlová, M., 2015. The effect of digestate, cattle slurry and mineral fertilization on the winter wheat yield and soil quality parameters. Plant Soil Environ. 61, 522–527. https://doi.org/10.17221/530/2015-PSE.

Six, J., Paustian, K., 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. Soil Biol. Biochem. 68, A4–A9. https://doi.org/10.1016/j.soilbio.2013.06.014.

Sofo, A., Ciarfaglia, A., Scopa, A., Camele, I., Curci, M., Crecchio, C., Xiloyannis, C., Palese, A.M., 2014. Soil microbial diversity and activity in a Mediterranean olive orchard using sustainable agricultural practices. Soil Use Manage. 30, 160-167. http://doi.org/10.1111/sum.12097.

Soil Survey Staff, 2010. Keys to Soil Taxonomy, 11th ed. USDA-NRCS, Washington D.C.

Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston C.T., Sumner, M.E., 1996. Methods of Soil Analysis: Chemical Methods. Part 3. No. 5 in the SSSA Book Series. SSSA-ASA, Madison, WI.

Tambone, F., Scaglia, B., D’Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81, 577-583. https://doi.org/10.1016/j.chemosphere.2010.08.034.

Tambone, F., Adani, F., 2017. Nitrogen mineralization from digestate in comparison to sewage sludge, compost and urea in a laboratory incubated soil experiment. J. Plant Nutr. Soil Sci. 180, 355-365. https://doi.org/10.1002/jpln.201600241.

Teglia, C., Tremier, A., Martel, J.L., 2011. Characterization of solid digestates: Part 2, Assessment of the quality and suitability for composting of six digested products. Waste Biomass Valoris. doi: 10.1007/s12649-10-9059-x.

Vilanova Plana, P., Noche, B., 2016. A review of the current digestate distribution models: storage and transport. Waste Manag. Environ. 1, 345-357. https://doi.org/10.2495/wm160311.

Voelkner, A., Holthusen, D., Ellerbrock, R.H., Horn, R., 2015. Quantity of hydrophobic functional CH-groups-decisive for soil water repellency caused by digestate amendment. Int. Agrophysics 29, 247–255. https://doi.org/10.1515/intag-2015-0024.

Wezel, A., Soboksa, G., McClelland, S., Delespesse, F., Boissau, A., 2015. The blurred boundaries of ecological, sustainable, and agroecological intensification: a review. Agron. Sustain. Dev. 35, 1283-1295. https://doi.org/10.1007/s13593-015-0333-y.

Zdruli, P., Kapur, S., Çelik, I., 2010. Soils of the mediterranean region, their characteristics, management and sustainable use, in: Kapur, S., Eswaran, H., Blum, W. (Eds.), Sustainable Land Management. Springer, Berlin, pp. 125-142.

Zheng, H., Liu, W., Zheng, J., Luo, Y., Li, R., Wang, H., Qi, H., 2018. Effect of long-term tillage on soil aggregates and aggregate-associated carbon in black soil of Northeast China. PLoS One 13, e0199523. https://doi.org/10.1371/journal.pone.0199523.

Zuber, S.M., Villamil, M.B., 2016. Meta-analysis approach to assess effect of tillage on microbial biomass and enzyme activities. Soil Biol. Biochem. 97, 176-187. https://doi.org/10.1016/j.soilbio.2016.03.011.

Figure captions

Fig. 1. Overview of the two experimental sites: the olive orchard nearby Lamezia Terme (orange frame) and the citrus orchard nearby Locri (purple frame). Onset tables show geographic coordination reference, mean annual temperature and rainfall, major soil data and soil taxonomy.

Fig. 2. Changes in the soil aggregate stability index (ISS) (mean ± SD, *n=4*) in the olive and citrus orchard soils following the treatments (NT, DIG, TILL as in M&M) at five sampling times (6 days before (Pre-Treat) and then 2 days (T1), 7 weeks (T2), 18 weeks (T3) and one year (T4) after the treatment event) during the 2016/2017 cropping season. Within each sampling period, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

Fig. 3. Changes in soil electrical conductivity (EC) and pH (mean ± SD, *n=4*) in the olive and citrus orchard soils following the treatments (NT, DIG, TILL as in M&M) at five sampling times (6 days before (Pre-Treat) and then 2 days (T1), 7 weeks (T2), 18 weeks (T3) and one year (T4) after the treatment event) during the 2016/2017 cropping season. Within each sampling period, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

Fig. 4. Changes in total soil organic C (C_{org}) and N (TN) (mean ± SD, *n=4*) in the olive and citrus orchard soils following the treatments (NT, DIG, TILL as in M&M) at five sampling times (6 days before (Pre-Treat) and then 2 days (T1), 7 weeks (T2), 18 weeks (T3) and one year (T4) after the treatment event) during the 2016/2017 cropping season. Within each sampling period, different letters indicate significant differences among soil treatments (Tukey's HSD test at, $P < 0.05$).

Fig. 5. Changes in soil microbial biomass C (MBC) and MBC:C_{org} ratio (mean ± SD, *n=4*) in the olive and citrus orchard soils following the treatments (NT, DIG, TILL as in M&M) at five

sampling times (6 days before (Pre-Treat) and then 2 days (T1), 7 weeks (T2), 18 weeks (T3) and one year (T4) after the treatment event) during the 2016/2017 cropping season. Within each sampling period, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

Fig. 6. Changes in soil basal respiration (R_{bas}), metabolic quotient (qCO_2) and mineralization coefficient (qM) (mean ± SD, $n=4$) in the olive and citrus orchard soils following the treatments (NT, DIG, TILL as in M&M) at five sampling times (6 days before (Pre-Treat) and then 2 days (T1), 7 weeks (T2), 18 weeks (T3) and one year (T4) after the treatment event) during the 2016/2017 cropping season. Within each sampling period, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

Fig. 7. Changes in soil ammonium-N (NH_4^+-N), nitrate-N (and NO_3^--N), extractable organic N (EON) and total soluble N (TSN) (mean ± SD, $n=4$) in the olive and citrus orchard soils following the treatments (NT, DIG, TILL as in M&M) at five sampling times (6 days before (Pre-Treat) and then 2 days (T1), 7 weeks (T2), 18 weeks (T3) and one year (T4) after the treatment event) during the 2016/2017 cropping season. Within each sampling period, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

Fig. 8. Changes in soil microbial N (MBN) and potentially mineralizable N (PMN) (mean ± SD, $n=4$) in the olive and citrus orchard soils following the treatments (NT, DIG, TILL as in M&M) at five sampling times (6 days before (Pre-Treat) and then 2 days (T1), 7 weeks (T2), 18 weeks (T3) and one year (T4) after the treatment event) during the 2016/2017 cropping season. Within each sampling period, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

Table 1 Chemical, biochemical and microbiological properties of the solid fraction of the biogas digestate. Values are means ± SD (*n=3*) expressed on a dry matter basis

Parameter	Value
Chemical analyses	
pH [a]	8.77±0.01
EC (dS m^{-1} at 25°C) [b]	2.14±0.01
Dry matter (% fresh weight)	18.0±0.49
Ash (%)	14.4±0.16
Volatile solids (%)	85.6±0.16
Tot-C (g kg^{-1})	389.6±0.8
Tot-N (g kg^{-1})	16.02±0.70
C/N	24.3±1.5
NH_4^+-N (g kg^{-1})	5.59±0.47
NH_4^+-N (% Tot-N)	34.9
NO_3^--N (g kg^{-1})	0.034±0.002
Tot-polyphenols (mg g^{-1}) [c]	1.62±0.05
P (g kg^{-1})	1.24
K (g kg^{-1})	2.25
S (g kg^{-1})	0.218
Ca (g kg^{-1})	0.971
Mg (g kg^{-1})	0.789
Cl (g kg^{-1})	0.180
Fe (mg kg^{-1})	55.0
Mn (mg kg^{-1})	53.0
B (mg kg^{-1})	9.0
Cd (mg kg^{-1})	<0.01
Cr_{VI} (mg kg^{-1})	0.97
Pb (mg kg^{-1})	0.07
Ni (mg kg^{-1})	1.26
Hg (mg kg^{-1})	<0.1
Cu (mg kg^{-1})	1.92
Zn (mg kg^{-1})	25.2
Microbiological analyses	
Salmonella spp. (MPN 25 g^{-1})	Absent
Escherichia coli (CFU g^{-1})	$< 10^3$

[a] in a biomass:water (3:50, w/v) mixture

[b] in a biomass:water (1:10, w/v) mixture

[c] as gallic acid, determined by Folin Ciocalteu's reagent method

Table 2 Main physical and chemical properties of tested soils from the two study sites. Values are means ± SD (*n=3*) expressed on a dry matter basis

Soil variable	Study site	
	Olive orchard	Citrus orchard
Coarse sand (%)	6.6 ± 0.1	23.7 ± 0.7
Fine sand (%)	12.3 ± 0.3	34.0 ± 0.8
Coarse silt (%)	13.6 ± 0.3	17.3 ± 0.3
Fine silt (%)	22.5 ± 0.3	12.5 ± 0.3
Clay (%)	45.0 ± 0.8	12.5 ± 0.6
Texture (according to USDA)	Clay	Sandy loam
Bulk density (g cm^{-3})	1.48 ± 0.02	1.22 ± 0.14
Structural stability index (%)	73.9 ± 7.5	66.9 ± 1.1
pH_{H_2O}	5.44 ± 0.11	7.46 ± 0.12
$EC_{1:2}$ (dS m^{-1})	0.170 ± 0.013	0.210 ± 0.087
Total $CaCO_3$ (g kg^{-1})	0	22.5 ± 3.0
Active $CaCO_3$ (g kg^{-1})	0	6.9 ± 0.1
CEC ($cmol_+$ kg^{-1})	51.9 ± 2.4	36.1 ± 1.2
C_{org} (g kg^{-1})	21.30 ± 3.24	13.74 ± 0.15
N_t (g kg^{-1})	2.03 ± 0.29	1.03 ± 0.05
C/N	10.51 ± 0.35	13.34 ± 0.66
NH_4^+ - N (mg kg^{-1})	3.2 ± 0.2	5.1 ± 1.0
NO_3^- - N (mg kg^{-1})	2.8 ± 2.0	2.2 ± 1.3
Olsen-P (mg kg^{-1})	22.9 ± 2.2	20.4 ± 2.1

Table 3 Significant effects due to soil type, time, soil management and their interactions on the variability of physical (ISS) and chemical (EC, pH, C_{org}, TN) soil variables. Values are *P*-values from three-way ANOVA (Soil × Time × Management) and two-way ANOVA (Time × Management).

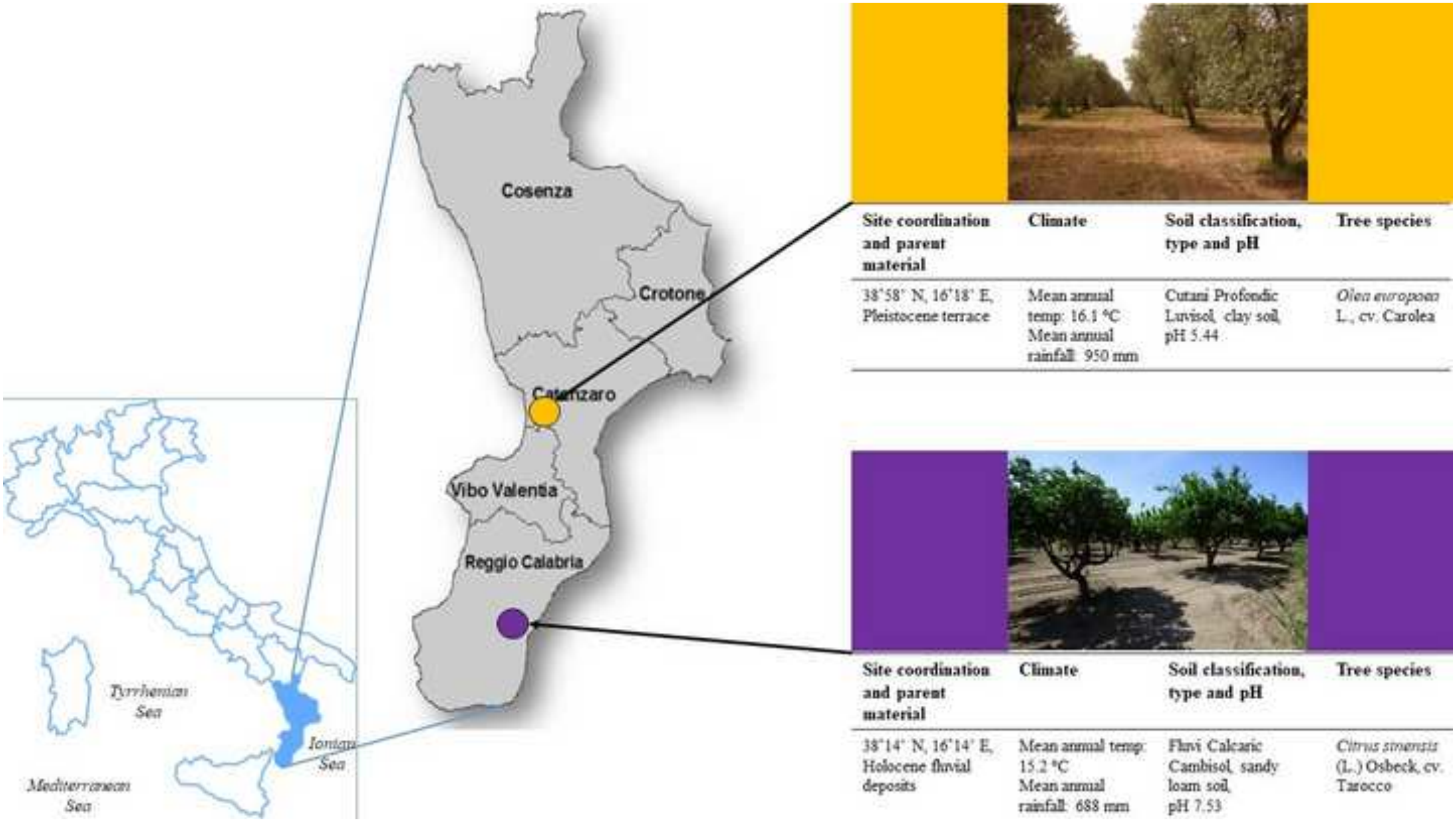
	ISS	EC	pH	C_{org}	TN
Three-way ANOVA					
Soil (S)	<0.001	<0.001	<0.001	<0.001	<0.001
S × T	0.007	<0.001	<0.001	<0.001	0.026
S × M	0.112	0.009	0.003	<0.001	0.018
S × T × M	0.021	0.001	0.042	0.055	0.118
Two-way ANOVA					
Time (T)	0.396	<0.001	0.826	0.472	0.239
Management (M)	<0.001	<0.001	0.987	<0.001	0.001
T × M	0.027	<0.001	1.000	0.962	0.781

Table 4 Significant effects due to soil type, time, soil management and their interactions on the variability of C-related biochemical (MBC, MBC:C_{org}, R_{bas}, $q\mathrm{CO}_2$, qM) soil variables. Values are *P*-values from three-way ANOVA (Soil × Time × Management) and two-way ANOVA (Time × Management).

	MBC	MBC:C_{org}	R_{bas}	$q\mathrm{CO}_2$	qM
Three-way ANOVA					
Soil (S)	0.156	<0.001	0.001	<0.001	<0.001
S × T	0.001	<0.001	<0.001	<0.001	<0.001
S × M	0.003	0.002	<0.001	0.001	0.001
S × T × M	0.073	0.001	<0.001	<0.001	0.001
Two-way ANOVA					
Time (T)	<0.001	0.001	<0.001	<0.001	<0.001
Management (M)	<0.001	0.276	<0.001	<0.001	<0.001
T × M	0.001	0.846	<0.001	0.088	0.004

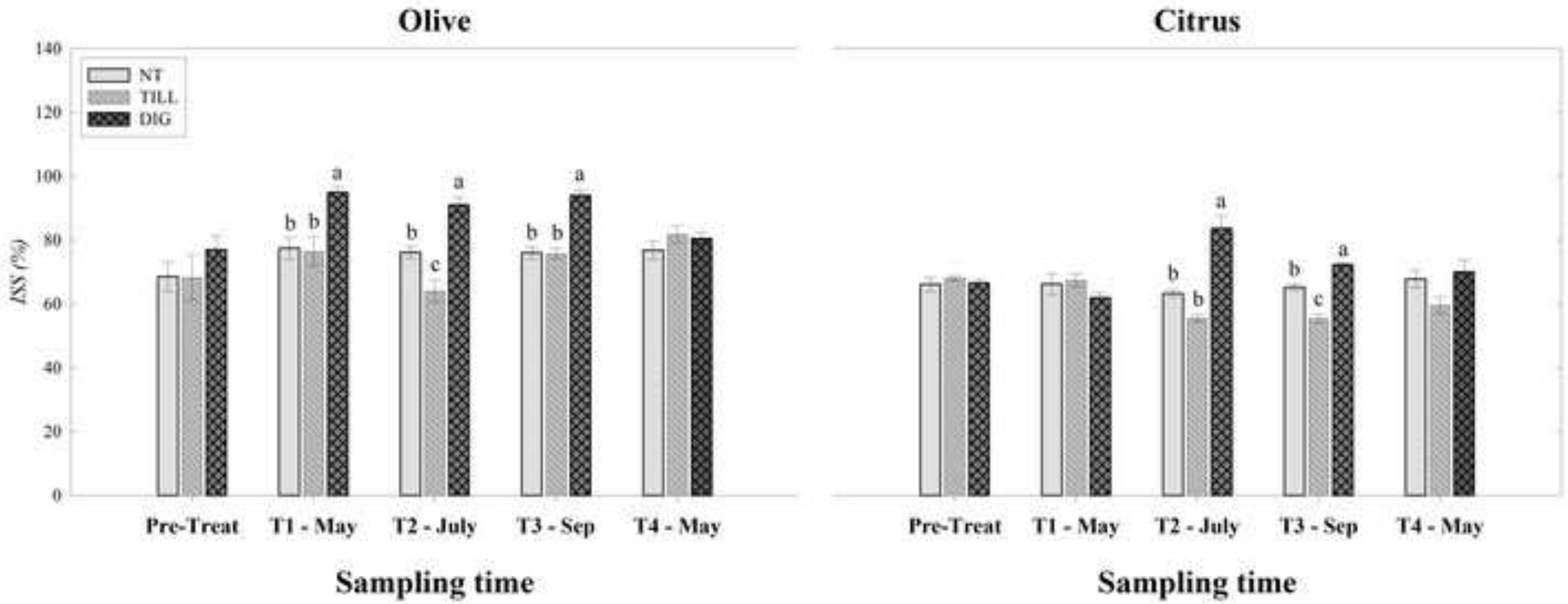
Table 5 Significant effects due to soil type, time, soil management and their interactions on the variability of N-related chemical (NH_4^+-N, NO_3^--N, EON, TSN) and biochemical (MBN, PMN) soil variables. Values are *P*-values from three-way ANOVA (Soil × Time × Management) and two-way ANOVA (Time × Management).

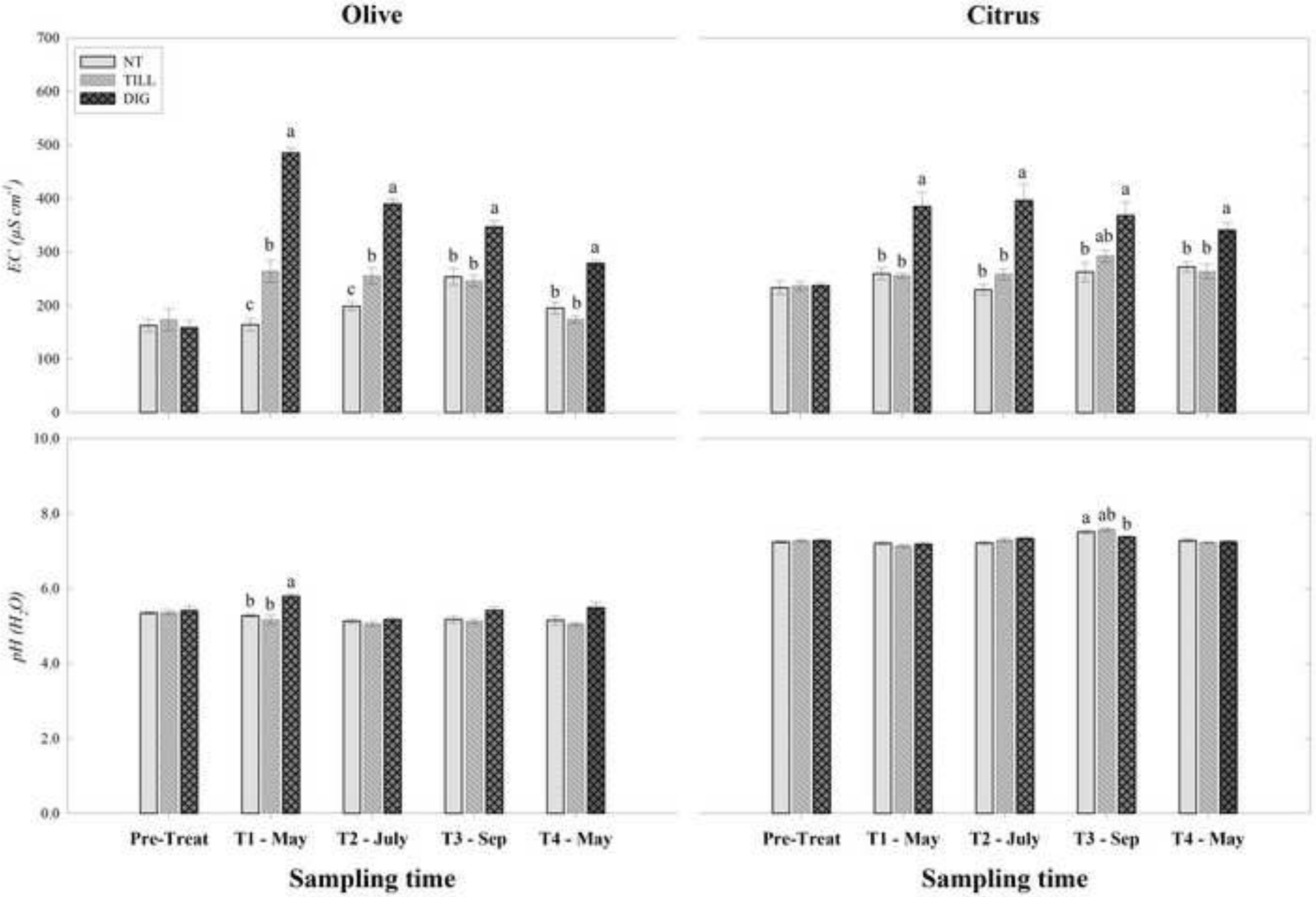
	NH_4^+-N	NO_3^--N	EON	TSN	MBN	PMN
Three-way ANOVA						
Soil (S)	<0.001	0.031	<0.001	<0.001	<0.001	<0.001
S × T	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
S × M	<0.001	0.084	0.004	0.001	<0.001	0.001
S × T × M	<0.001	<0.001	0.068	0.005	0.006	0.001
Two-way ANOVA						
Time (T)	<0.001	<0.001	<0.001	<0.001	<0.001	0.006
Management (M)	<0.001	<0.001	0.002	<0.001	0.001	0.001
T × M	<0.001	<0.001	0.006	<0.001	0.581	0.007

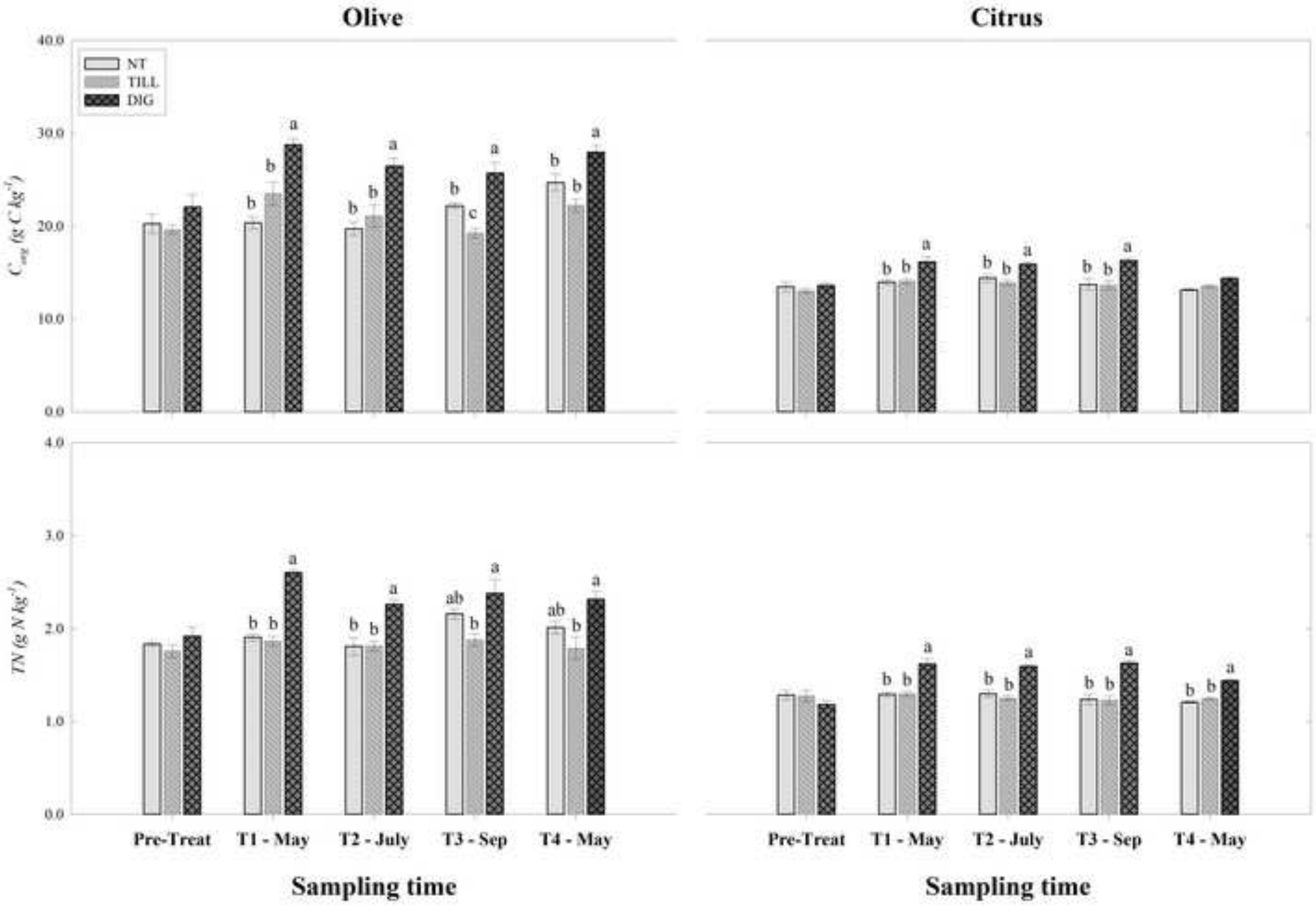


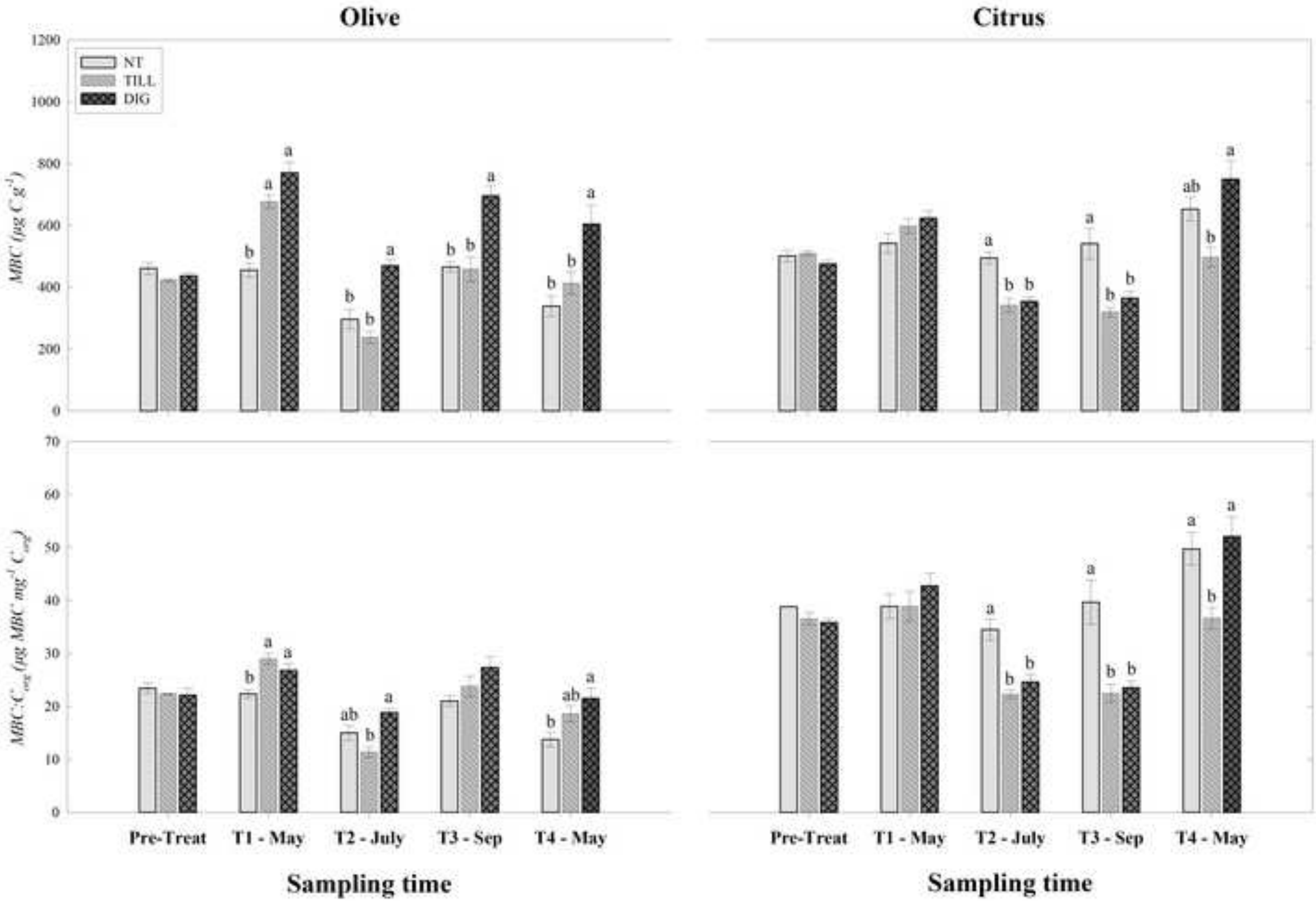
Site coordination and parent material	Climate	Soil classification, type and pH	Tree species
38°58' N, 16°18' E, Pleistocene terrace	Mean annual temp: 16.1 °C Mean annual rainfall: 950 mm	Cutani Profondic Luvisol, clay soil, pH 5.44	*Olea europaea* L., cv. Carolea

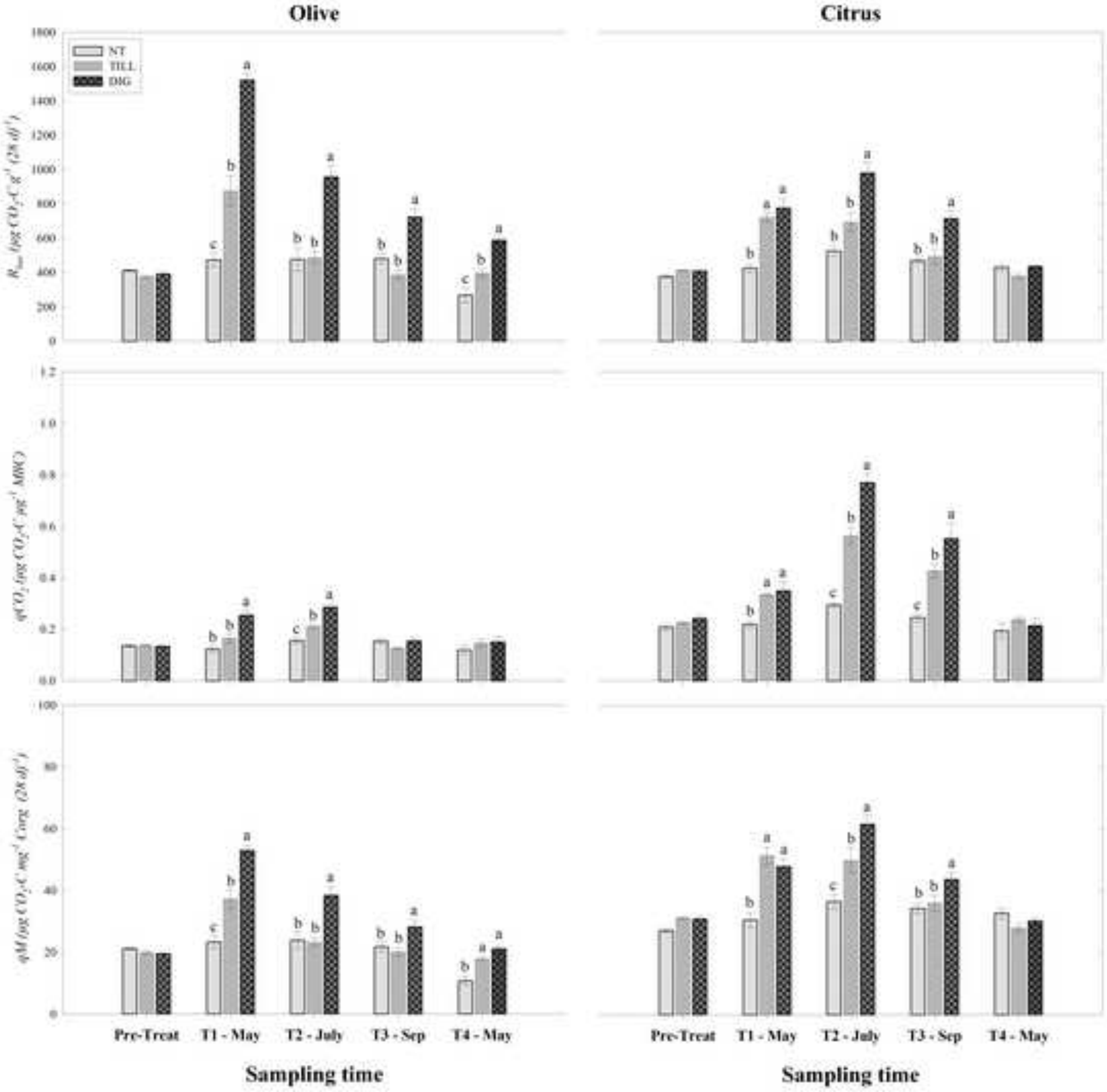
Site coordination and parent material	Climate	Soil classification, type and pH	Tree species
38°14' N, 16°14' E, Holocene fluvial deposits	Mean annual temp: 15.2 °C Mean annual rainfall: 688 mm	Fluvi Calcaric Cambisol, sandy loam soil, pH 7.53	*Citrus sinensis* (L.) Osbeck, cv. Tarocco

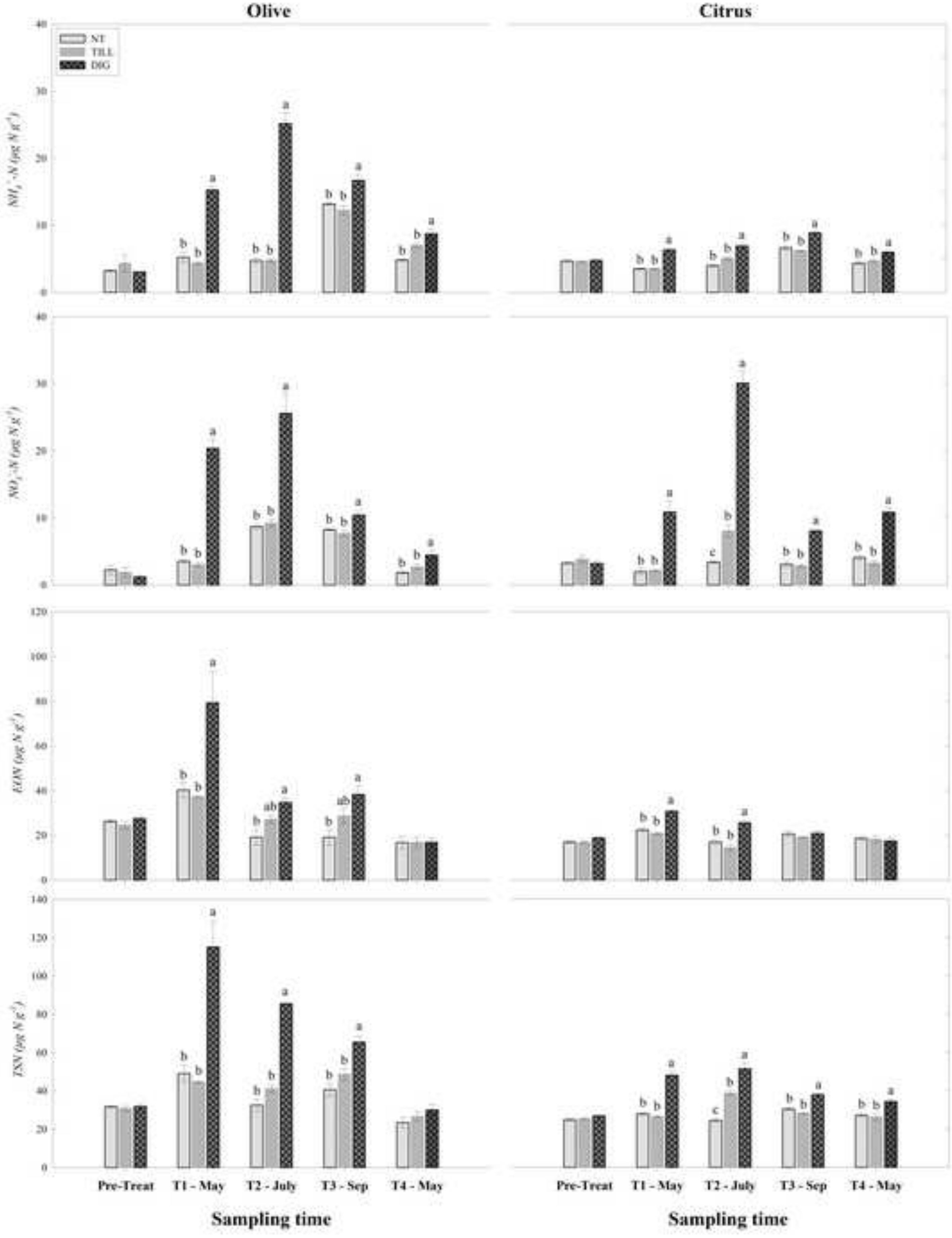

Olive
Citrus
140
120
100
80
60
40
20
0
ISS (%)
NT
TILL
DIG
a
b b a
b a
c
b b a
a
b
b
b
a
c
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time


Olive
Citrus
NT
TILL
DIG
EC (µS cm⁻¹)
0
100
200
300
400
500
600
700
pH (H₂O)
0.0
2.0
4.0
6.0
8.0
10.0
a
b
c
c
b
a
b
b
a
b
b
a
b
b
a
b
b
a
b
ab
a
b
b
a
b
b
a
b
a
ab
b
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time

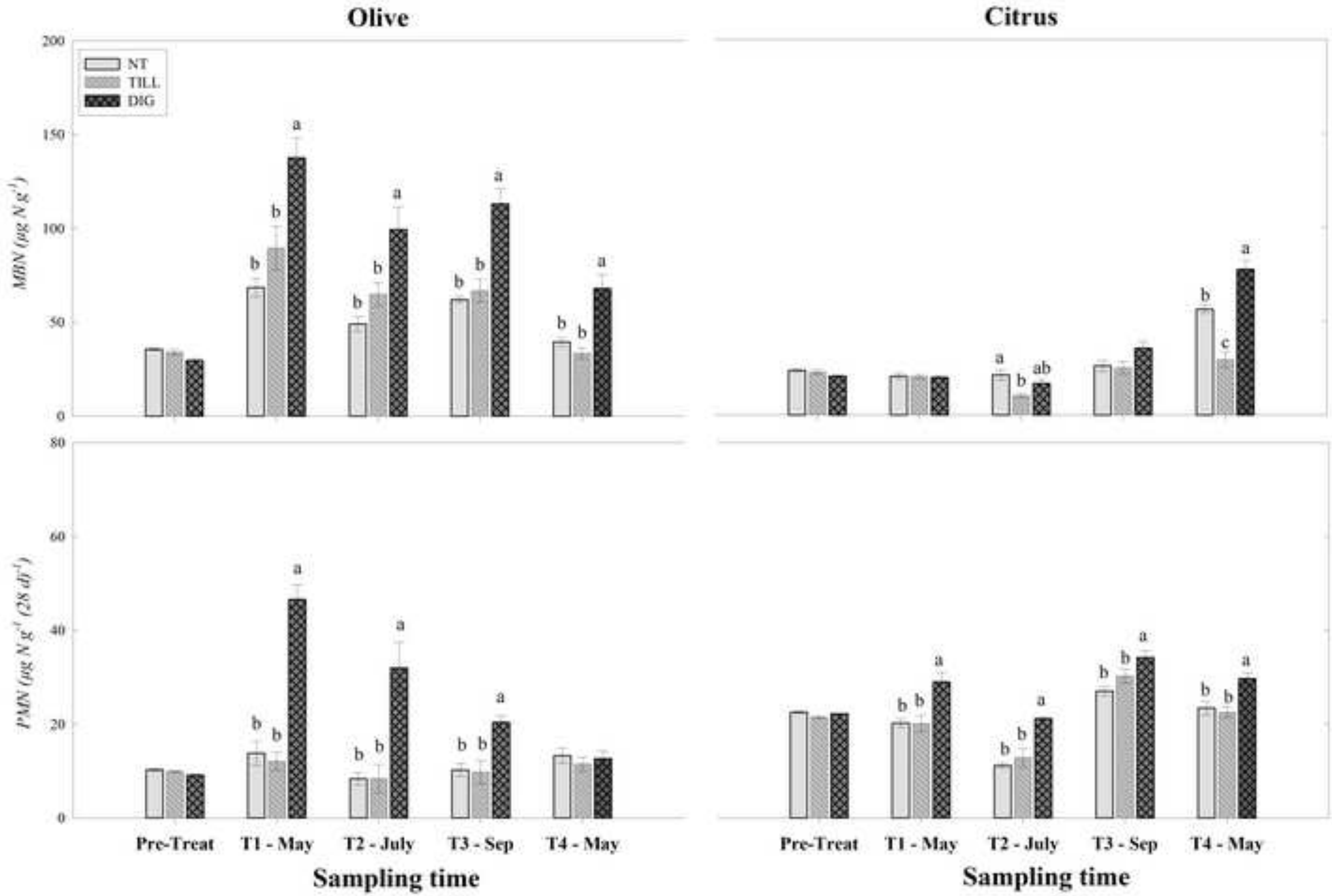

Olive
Citrus
NT
TILL
DIG
C_{org} (g C kg^{-1})
TN (g N kg^{-1})
40.0
30.0
20.0
10.0
0.0
4.0
3.0
2.0
1.0
0.0
b
b
a
b
b
a
b
c
a
b
b
a
ab
b
a
ab
b
a
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time
Sampling time


Olive
Citrus
NT
TILL
DIG
MBC (μg C g⁻¹)
MBC:Corg (μg MBC mg⁻¹ Corg)
0
200
400
600
800
1000
1200
0
10
20
30
40
50
60
70
a
b
ab
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time
Sampling time


Olive
Citrus
NT
TILL
DIG
R_{bas} (μg CO_2-C g^{-1} (28 d)$^{-1}$)
1800
1600
1400
1200
1000
800
600
400
200
0
a
b
c
a
b
b
a
b
b
a
b
c
a
a
b
a
b
b
a
b
b
qCO_2 (μg CO_2-C μg^{-1} MBC)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
b
b
a
b
c
a
a
b
a
b
c
a
b
c
qM (μg CO_2-C mg^{-1} Corg (28 d)$^{-1}$)
100
80
60
40
20
0
a
b
c
a
b
b
a
b
b
a
a
b
a
a
b
a
b
c
a
b
b
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time



Olive
Citrus
NT
TILL
DIG
NH4+-N (µg N g-1)
NO3--N (µg N g-1)
EON (µg N g-1)
TSN (µg N g-1)
0
10
20
30
40
0
20
40
60
80
100
120
140
a
b
ab
c
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time
Sampling time


Olive
Citrus
NT
TILL
DIG
MBN (μg N g-1)
PMN (μg N g-1 (28 d)-1)
0
50
100
150
200
0
20
40
60
80
b
b
a
b
b
a
b
b
a
b
b
a
a
b
ab
b
c
a
b
b
a
b
b
a
b
b
a
b
b
a
b
b
a
b
b
a
b
b
a
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time
Pre-Treat
T1 - May
T2 - July
T3 - Sep
T4 - May
Sampling time