Cleaner and Circular Bioeconomy 1 (2022) 100004

Contents lists available at ScienceDirect

Cleaner and Circular Bioeconomy

journal homepage: www.elsevier.com/locate/clcb

Short-term effects of repeated application of solid digestate on soil C and N dynamics and CO_2 emission in a clay soil olive (*Olea europaea* L.) orchard

Giuseppe Badagliacca, Maurizio Romeo, Antonio Gelsomino, Michele Monti*

Dipartimento di Agraria, Università degli Studi Mediterranea di Reggio Calabria, Feo di Vito, Reggio Calabria 89124, Italy

ARTICLE INFO

Keywords:
Solid anaerobic digestate
Repeated amendment
Olea europaea L.
C and N dynamics
CO_2 soil emission
Digestate legacy

ABSTRACT

Olive orchards cultivation often has to face several soil degradation phenomena like organic matter depletion, reduction of biological activity and generalised loss of fertility. In these contexts, agricultural solid digestate has shown promising results in restoring fertility, although its sustainability has not been fully assessed. Therefore, to evaluate its common use in this agroecosystem, we conducted a field experiment to study the short-term effects of repeated amendment with solid anaerobic digestate on the integral fertility of an olive orchard grown in a clayey soil in Southern Italy. We compare the following treatments: unamended control, one-year and two-year solid digestate amendment application. During the experiment, we assessed changes in soil fertility and dynamics of C and N pools by measuring a set of chemical, biochemical and soil eco-physiological variables and by monitoring CO_2 fluxes at field scale. Results showed growing benefits from single to repeated treatment with solid digestate, compared to the control, such as an increase of soil soluble C and N forms, organic matter and microbial pools with higher microbial activity and, despite a relative increase in CO_2 field emission, microbial efficiency, thus proving to be a sustainable management for olive orchard agroecosystems.

Introduction

Olive tree cultivation for the production of table olives and olive oil in the Mediterranean basin represents one of the most important perennial cropping systems, covering about ~10 Mha and generating an economic volume of about ~€ 8.3 billion (FAOSTAT 2020). Olive cultivation in many cases is affected by several issues closely linked to soil degradation processes such as erosion, organic matter depletion, loss of biodiversity and diffuse pollution (Gómez-Limón et al., 2012; López-Pintor et al., 2018) that can negatively affect the provision of agroecosystem services, going as far as desertification (Karamesouti et al., 2015; González-Rosado et al., 2021). Moreover, in these contexts, conventional management practices do nothing to counteract these processes or they even stimulate degradation (Sastre et al., 2017; Vignozzi et al., 2019).Therefore, it is necessary to adopt alternative management systems to support production, making it more sustainable by improving soil quality and reducing Greenhouse gases (GHG) emissions (González-Rosado et al., 2020; Montanaro et al., 2021). With this aim, different authors (e.g.: Scotti et al., 2015; Gómez-Sagasti et al., 2018) have proposed the use of organic amendments to prevent and halt soil degradation in tree crops.

Among organic matrices, solid anaerobic digestate, a by-product from biogas plant, has attracted a growing interest from farmers as a soil conditioner and a replacement for synthetic fertilisers because of its organic matter content (~40%) and relative abundance of nutrients such as ammonium-N (NH_4^+-N), phosphorus (P) and potassium (K) (Insam et al., 2015; Tsachidou et al., 2019). However, applying this organic amendment could affect several chemical and biochemical soil parameters. Solid digestate can increase the pH (Cardelli et al., 2018) and electrical conductivity (EC) (Barłóg et al., 2020); while, the addition of a large quantity of NH_4^+-N, that can be speedily nitrified, can lead to an uncontrolled release of nitrate-N (NO_3^--N) available for crops but is also highly exposed to the risk of leaching (Makádi et al., 2012; Reuland et al., 2021). In addition, supplying readily available carbon (C) digestate might affect the C budget, promote microbial biomass and can trigger microbial respiration increasing soil CO_2 emissions (Grigatti et al., 2020; Egene et al., 2021; Holatko et al., 2021). However, microbial responses to solid digestate amendment are variable and contrasting results have been reported (Johansen et al., 2013; Makádi et al., 2012).

According to Weiland (2010), about 23 m^3 of digestate per kW installed are produced yearly while Corden et al. (2019) estimated an amount of 180 million tonnes at the European level, of which 30 million tonnes are produced in Italy, with anticipated increases for the future. Considering that production is continuous throughout the year, the bulky nature of this by-product and its relatively high transportation cost, approaches that promote local reuse and nutrient recycling within a circular economy strategy should be implemented in adjacent areas close to biogas plants (Tamburini et al., 2020). Therefore, in these

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* Corresponding author.
E-mail address: montim@unirc.it (M. Monti).

https://doi.org/10.1016/j.clcb.2022.100004
Received 7 December 2021; Received in revised form 20 January 2022; Accepted 30 January 2022

agronomical contests the effects of repeated digestate application over time should be seriously evaluated but field research remains scarce.

Badagliacca et al. (2020a) have shown that a single application of solid anaerobic digestate into clay soil is capable of a long-lasting increase in organic carbon and nitrogen pools, with positive effects on structure and microbial carbon use efficiency, restoring the fertility of an olive orchard soil under a Mediterranean climate. Thus, starting from these observations, in order to assess the short-term effects (over two years) of intensive use (yearly) of digestate, the present study tested the effect of repeated solid digestate amendment, compared to a single application and unamended control, on the fertility of an olive orchard soil by monitoring chemical and biochemical properties, eco-physiological indices and in-field CO_2 emission.

Materials and methods

Experimental site

The trial was established under rainfed conditions during the 2016/18 growing seasons in an olive orchard (*Olea europaea* L. cv. *Carolea*) in Southern Italy, within the Tyrrhenian side of the Calabria region, near Lamezia Terme, Catanzaro, Italy (38˚58’ N, 16˚18’ E, 81 m a.s.l.). The climate of the experimental site is mild and rainy in winter and warm and dry in summer with a mean annual rainfall of 1094 mm and mean air temperature of +14.3 ˚C (1985–2015 average) (ARPACAL, 2018). The experimental field had been continuously cultivated since the mid-50 s (70-year-old plants) with olive trees spaced at a distance of 6 × 6 m and periodically tilled. The soil is acid clayey and is classified according to USDA as a Typic Hapludalf fine, mixed thermic (Soil Survey Staff, 2010), while it is a Cutanic Profondic Luvisol according to IUSS (IUSS Working Group WRB, 2006), with a thermic thermal regime and udic moisture regime, respectively (ARSSA, 2003). Main soil properties were: 18.9% sand, 36.1% silt, 45.0% Clay, Clay texture (USDA), pH 5.44 (1:2.5 H_2O), EC 0.170 dS m^{-1}, CEC 51.9 $cmol_+$ kg^{-1}, total organic C 21.30 g kg^{-1}, total N 2.03 g kg^{-1}. Further information regarding the soil is available in Supplementary Table 1.

2.2. Solid anaerobic digestate

Agricultural solid anaerobic digestate was produced by a medium-scale (999 kWe) continuous mesophilic (T ~40 ˚C) biogas producing plant, adjacent to the experimental field, supplied with zootechnical effluents (cow and poultry) (70%), crop residues from pruning materials, citrus pomace, olive mill wastewater and pomace (20%), and dairy wastewater (10%). The resultant digestate was separated into two fractions as usual, the liquid digestate, which was discarded, and the solid digestate that was used in this experiment. The main characteristics of the solid anaerobic digestate were: Dry matter 18.0%, pH 8.77, EC 2.14 dS m^{-1}, Ash 14.4%, volatile solids 85.6%, total C 389.6 g kg^{-1}, total N 16.02 g kg^{-1}, C/N 24.3, $NH_4{}^+$-N 5.59 g kg^{-1}, $NO_3{}^-$-N 0.034 g kg^{-1}, total polyphenols 1.62 mg g^{-1}, P 1.24 g kg^{-1}, K 2.25 g kg^{-1}. A detailed description of the solid anaerobic digestate is available in Supplementary Table 2.

2.3. Experimental design and soil managements

The experimental set-up at the end of March 2017 comprised field plots (20 m × 12 m each) laid out in a randomised complete block design (RCBD), with four replications, in order to test the following treatments: (1) unamended control (CTR), which provided for an inter-row harrowing (~ 20 cm) followed by a slight rolling; (2) amendment with solid digestate (DIG) at a rate of 30 t ha^{-1} (The common dose applied by the local farmers, consistently with Barra Caracciolo et al., 2015 and Badagliacca et al., 2020a) which was incorporated into the soil by harrowing (same as in CTR); (3) repeated solid digestate application (DIGP), where the digestate was further applied according to the same rate and procedure performed in DIG, for two consecutive years (the preceding cropping season 2016/17, i.e. 8 April 2016, plus the experimental year 2017/18, i.e. 5 April 2017).

Soil sampling

Soil samples were gathered during the 2017/18 growing season before (T0, end of March 2017) and then ~2 weeks (T1, early April 2017, shoot development BBCH 33), ~3 months (T2, late June 2017, fruit development BBCH 72) and ~1 year (T3, mid-April 2018, shoot development BBCH 31) after the application of the treatments. Nine individual soil cores per plot were taken in the inter-row between the plants, in order to minimize any plant effect, from 0 to 20 cm soil layer (directly affected by the digestate incorporation) then assembled and mixed to obtain a single composite sample. Four composite samples (replicates) were taken per treatment, twelve (3 treatments × 4 replicates) at each sampling time, forty-eight in total for the whole experiment. In the laboratory, each field sample was split into two sub-samples: an aliquot of moist field soil was processed within 24 h for biochemical analyses while the remaining part was air-dried, sieved with a 2 mm sieve, and then used for determining chemical variables.

Soil chemical and biochemical variables determination

Chemical soil properties were determined according to the methods described in Sparks et al. (1996). Soil pH was measured in a 1:2.5 (w/v) soil-to-0.01 M $CaCl_2$ solution mixture (pH_{CaCl2}) while EC was determined at 25 ˚C in a 1:2 (w/v) soil-to-water ratio mixture ($EC_{1:2}$ 25 ˚C). Total organic C (C_{org}) and N (TN) concentrations were measured by an elemental analyzer LECO CN628 (LECO Corporation, MI, USA). Soil $NH_4{}^+$-N and $NO_3{}^-$-N concentrations were determined by extracting samples with 2 M KCl (1:10, w/v) and analyzing extracts by Flow Injection Analysis System (FIAS 400 PerkinElmer, Inc., CT, USA). On 2 M KCl extracts, total soluble N (TSN) was determined by an elemental analyzer TOC-L_{CSH} Shimadzu (Shimadzu Corporation, Tokyo, J). Then, extractable organic N (EON) was obtained as the difference between the TSN and the sum of $NH_4{}^+$-N and $NO_3{}^-$-N.

Microbial biomass C and N (MBC and MBN) were measured by the chloroform fumigation-extraction method, quantifying C and N concentration in soil extracts by a Shimadzu TOC-LCSH elemental analyzer. Soil basal respiration (R_{bas}) was measured accounting for total CO_2-C emission during a 28-day incubation time, according to Öhlinger et al. (1995), using the Shimadzu TOC-LCSH elemental analyzer. Potentially mineralisable N (PMN), was estimated as the soil inorganic-N released after the 28 days of incubation time (related to R_{bas}) minus the inorganic soil N at day 0 (Drinkwater et al., 1996). Following Anderson (2003), eco-physiological indices such as microbial quotient (MBC:C_{org}), metabolic quotient (qCO_2) and mineralisation coefficient ($qM = R_{bas}:C_{org}$) were computed to assess the effect of treatments on microbial functioning.

Field soil CO_2 fluxes and total emission

During the experiment (from April 2017 to May 2018) soil CO_2 fluxes were measured in field by LI-8100A automated soil CO_2 flux system infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA) equipped with a multiplexer unit LI-8150 (LI-COR Inc.) connected to long-term chambers LI-8100–104 (LI-COR Inc.) for continuous field measurements. A chamber per plot was installed in the inter-row between two plants, so as to minimize any root respiration interference. Shortly after the establishment of the treatments, to avoid CO_2 flux underestimation due to lateral diffusion from the soil column, PVC collars with a 20.3 cm diameter (317.8 cm^2 covered surface) were inserted into the soil to a depth of 5 cm. During the measurement, a double gasket installed in the Long-term Chamber LI-8100–104 ensured the sealing between collar and chamber minimizing CO_2 leaks and wind interference. The

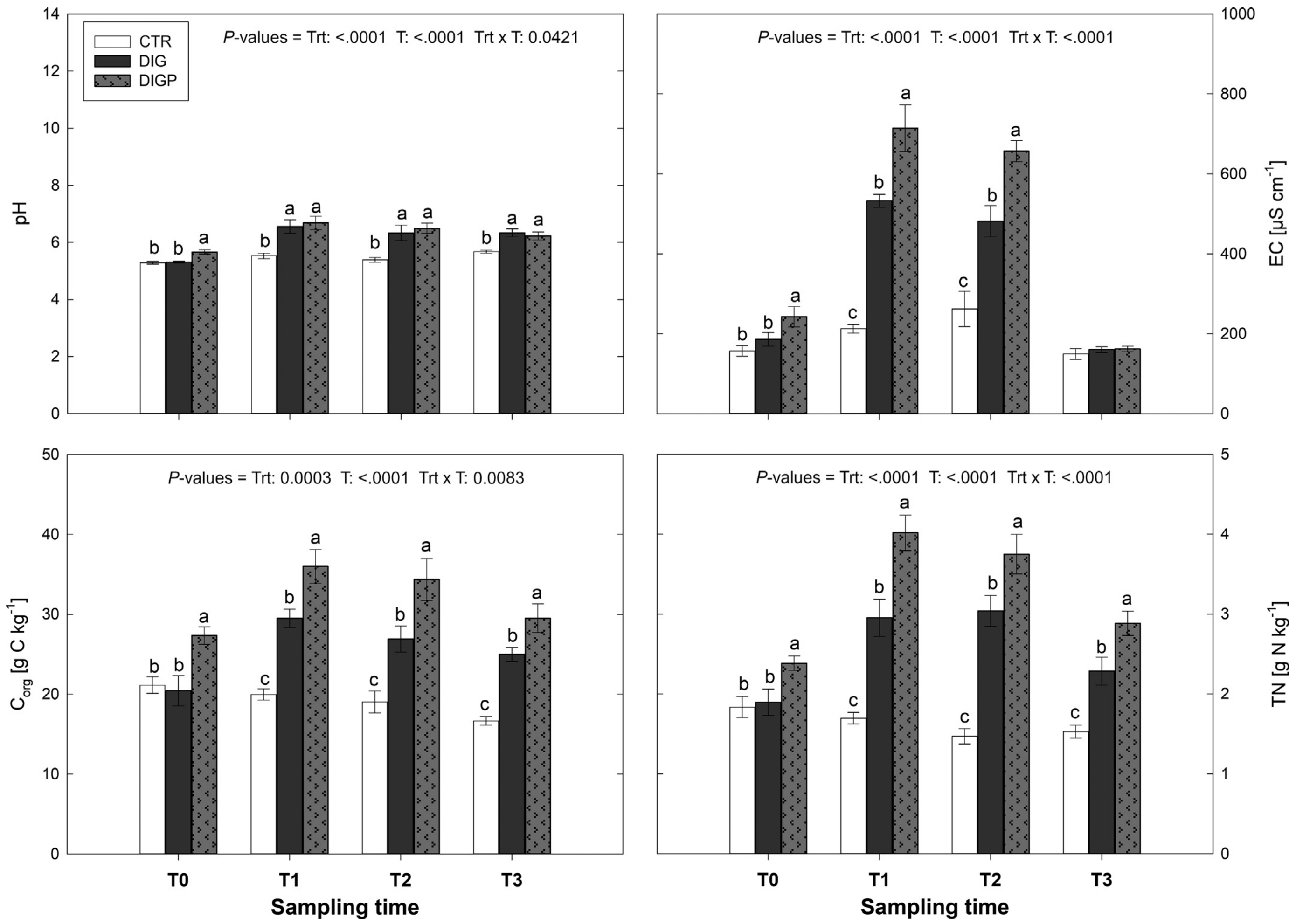

Fig. 1. Soil pH, electrical conductivity (EC), total organic C (C_{org}) and total-N (TN) concentrations (mean ± SD, $n = 4$) in the olive orchard soil following the treatments (CTR, DIG, DIGP) at four sampling times (before (T0) and then ~2 weeks (T1), ~3 months (T2) and ~1 year (T3) after the treatment application) during the 2017/2018 cropping season. Within each sampling time, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

observation time was 120 s to minimize chamber CO_2 concentration changes during analysis and a 30 s dead band was programmed to allow for equilibration of the chamber pressure upon closure. Soil CO_2 fluxes were measured every 6 h to assess variation throughout the day. Volumetric soil moisture (by ECH_2O Model EC-5, Decagon Devices Inc., Pullman, WA, USA) and temperature (by Licor 8100–203 Soil Temperature Thermistor, LI-COR Inc., Lincoln, NE, USA) were monitored during the experiment. CO_2 emission fluxes were computed by LI-8100 Data File Viewer ver. 3.0 (LI-COR Inc., Lincoln, NE, USA). The total amount of CO_2 emitted was calculated by using the following equation proposed by Cheng et al. (2012):

$$Total\ CO_2\ emission\ \left[g\ CO_2\ m^{-2}\right] = \sum_{i=1}^{n} \left(F_i + F_{i+1}\right)/2\ \times \left(t_{i+1} - t_i\right) \times\ 24 \tag{1}$$

where F is the CO_2 flow at the *ith* measurement, (*ti+1-ti*) is the time length between two adjacent measurements and n is the total measurement number.

Statistics

Soil properties data were reported as mean values ($n = 4$) expressed on a dry weight (DW) basis (105 °C, 24 h). A two-way analysis of variance (ANOVA) (Treatment × Time) with repeated measures was performed to assess the effect of treatments, sampling time and their interaction on tested soil variables. Total soil CO_2 emission data were subjected to one-way ANOVA. Tukey HSD (honestly significant difference) test at $P < 0.05$ was used for pairwise multiple comparisons of treatments means within each sampling time and among cumulated CO_2 emission measured in the field. Statistical analyses were performed in SAS environment (SAS 9.3, SAS Institute, Cary, NC, USA) while graphs were plotted using SigmaPlot v10 (Systat Software Inc., San Jose, California, USA).

Results

Chemical parameters

Treatments, sampling times and their interactions significantly affected all soil chemical properties investigated (Trt: $P < 0.0001$, T: $P < 0.0001$, Trt × T:$P < 0.05$; Figs. 1 and 2). With regard to the pH, the preceding application in DIGP treatment lead to observe a slightly higher pH value (+7%), compared to the other treatments, already at the first sampling time (T0); then, digestate, both in first (DIG) and repeated (DIGP) application, increased values of this variable by 0.96 (+18%) during the whole experimental period (from T1-April 2017 to T3-April 2018) (Trt × T:$P = 0.0421$) (Fig. 1). In the same way, we observed the preceding amendment effect on EC, with DIGP showing higher values at T0 (+82.5 µS cm^{-1}, on average) compared to the other treatments. At the following sampling times (T1-April 2017 and T2-June 2017), di-

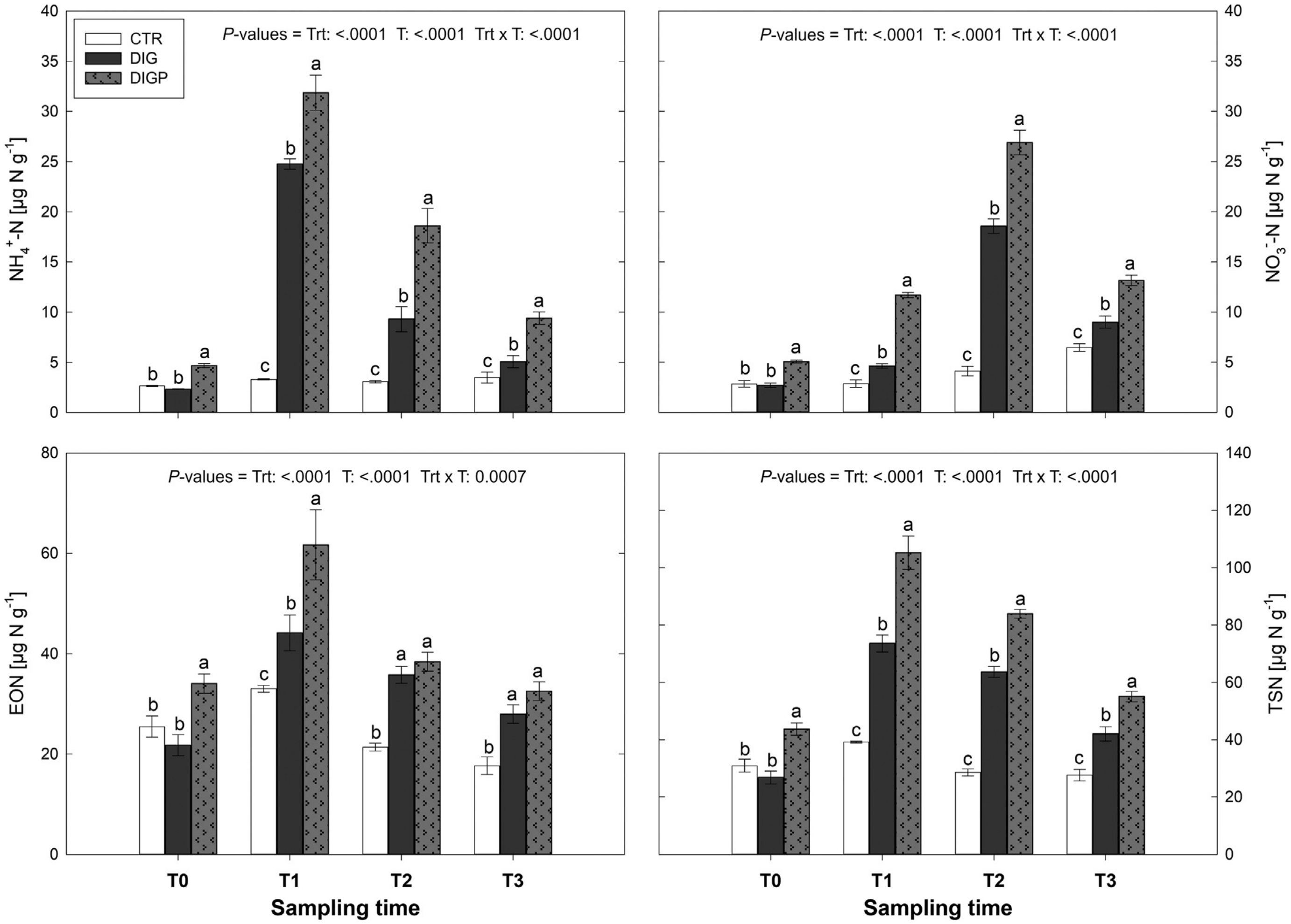

Fig. 2. Soil ammonium-N (NH_4^+-N), nitrate-N (NO_3^--N), extractable organic N (EON) and total soluble N (TSN) concentrations (mean ± SD, $n = 4$) in the olive orchard soil following the treatments (CTR, DIG, DIGP) at four sampling times (before (T0) and then ~2 weeks (T1), ~3 months (T2) and ~1 year (T3) after the treatment application) during the 2017/2018 cropping season. Within each sampling time, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

gestate strongly increased EC by +117% in DIG and +193% in DIGP, compared to the unamended control (CTR), reaching the highest values equal to 532 µS cm^{-1} in DIG and 714 µS cm^{-1} in DIGP. Then, no significant effects were found at the last sampling (T3-April 2018, one year later; Trt × T:$P < 0.0001$) (Fig. 1). Digestate positively affected soil organic matter, investigated by determining C_{org} and TN. In particular, preceding amendment (DIGP) showed a legacy effect on these two variables at T0 (+29%, on average). Afterwards, compared to T0, amendment increased the concentration of C_{org} and TN by +48% in DIG and +80% in DIGP, on average, at T1-April 2017 and T2-June 2017; one year after treatments application, at T3-April 2018, C_{org} and TN slightly decreased in DIG and DIGP, although they remained higher than CTR (+52% in DIG and +80% in DIGP, on average compared to CTR) (Trt × T:$P < 0.0083$ and $P < 0.0001$) (Fig. 1). In the unamended control (CTR), we found a small reduction of both variables along the sampling times (−14%, on average at the end of the experiment, T3-April 2018) (Fig. 1). Because of the amendment, all soluble N forms increased (Fig. 2). In particular, NH_4^+-N concentration from T0, where only the effect of the preceding amendment was detected in DIGP (+90% respect to the other treaments), showed a strong upward trend by 6.5-fold in DIG and 8.6-fold in DIGP at T1-April 2017 (reaching the highest values equal to 24.7 mg kg^{-1} in DIG and 31.9 mg kg^{-1} in DIGP), compared to CTR, before descending at the following sampling time (+202% for DIG and +504% for DIGP at T2-June 2017, compared to CTR) (Trt × T:$P < 0.0001$). At the last sampling (one year post-treatments application, T3-April 2018), a slight difference separated CTR and DIG treatments (+45% in DIG than in CTR) while NH_4^+-N concentration in DIGP was higher (+170%, on average among CTR and DIG) (Trt × T: $P < 0.0001$) (Fig. 2). In the same way, although more gradually and progressively, the soil NO_3^--N increased in both amended treatments (DIG and DIGP, compared to CTR), reaching the highest values at T2-June 2017 with the concentrations of 18.6 mg kg^{-1} for DIG and of 26.9 mg kg^{-1} for DIGP treatment. Then, at T3-April 2018, NO_3^--N concentrations decreased but maintaining significant differences between the treatments (+39% in DIG and +104% in DIGP, compared to CTR; Trt × T: $P < 0.0001$). The amendment also improved soil EON. In particular, DIG treatment increased this variable (compared to T0 level) by +103% at T1-April 2017 while then, in the subsequent samplings, its effect on EON concentration was lower and equal to +64% at T2-June 2017 and +29% at T3-April 2018 (Trt × T:$P = 0.0007$) (Fig. 2). The preceding application of amendment highlighted a residual effect on DIGP at T0 (+44%, on average compared to other treatments). Then, for this treatment, EON showed the highest concentration at T1-April 2017 (equal to 61.7 mg kg^{-1}; +161%), while in the subsequent sampling times its levels was equal to DIG (+63% at T2-June 2017 and +38% at T3-April 2018, compared to the mean T0 levels of CTR and DIG; Trt × T:$P = 0.0007$) (Fig. 2). As a consequence of the variation of the previously mentioned N variables due to amendment, soil TSN in-

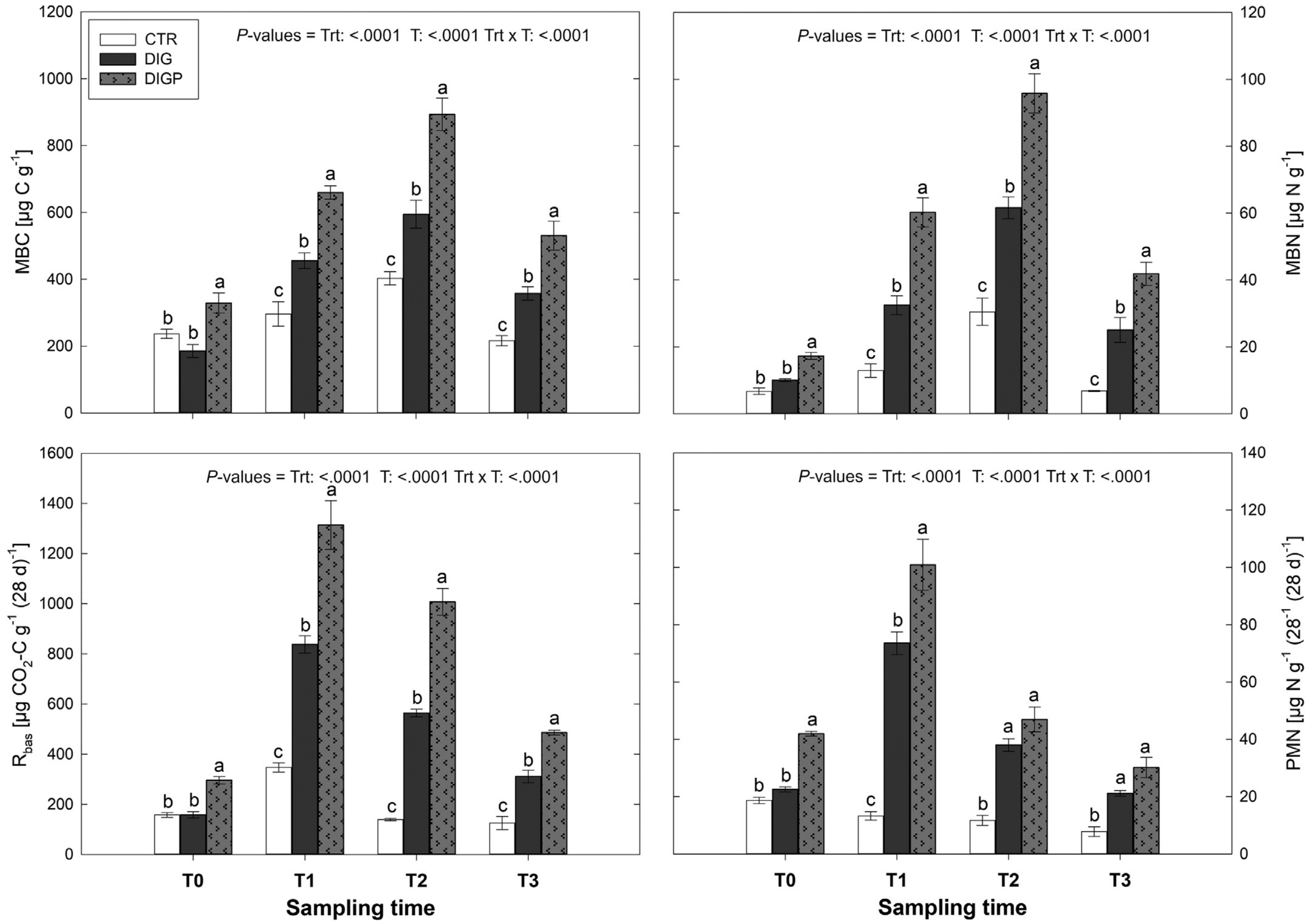

Fig. 3. Soil microbial biomass C (MBC), microbial biomass N (MBN), basal respiration (R_{bas}) and potentially mineralizable N (PMN) (mean ± SD, $n = 4$) in the olive orchard soil following the treatments (CTR, DIG, DIGP) at four sampling times (before (T0) and then ~2 weeks (T1), ~3 months (T2) and ~1 year (T3) after the treatment application) during the 2017/2018 cropping season. Within each sampling time, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

creased by +155%, +121%, +46% in DIG and +265%, +191%, +91% in DIGP, respectively, at T1-April 2017, T2-June 2017 and T3-April 2018, compared to CTR (Trt × T: $P < 0.0001$) (Fig. 2). Moreover, also in this case, the preceding amendment increased TSN values in DIGP already at T0 sampling by +51% compared with CTR and DIG (Fig. 2).

Biochemical parameters

Microbial C and N, R_{bas}, PMN and the related eco-physiological indices (MBC:C_{org}, qCO$_2$ and qM) show statistically significant effects from treatments, sampling times and their interactions (Trt: $P < 0.0001$, T: $P < 0.0001$, Trt × T: $P < 0.01$; Figs. 3 and 4). Microbial biomass C and N at T0 were higher in DIGP (+56% and +106% for MBC and MBN) than in the other treatments (CTR and DIG) (Fig. 3). After treatments application, at T1-April 2017 and T2-June 2017 the highest values of MBC and MBN were observed, with digestate amendment that increased both microbial biomass indicators according to this trend: CTR < DIG (+51% MBC, +127% MBN, on average among T1 and T2) < DIGP (+48% MBC, +71% MBN, on average among T1 and T2). Then, at the last sampling (one year later treatments application, T3-April 2018) both amended treatments showed higher values than the unamended control (CTR) with a stronger and long-lasting effect in DIGP (+145% MBC and +515% MBN compared to CTR) than in DIG (+65% MBC and +268% MBN compared to CTR) (Trt × T: $P < 0.0001$) (Fig. 3). The response of R_{bas} over the sampling times was similar to microbial biomass trend (Fig. 3). Indeed, at T0 the DIGP treatment showed higher values (+88%, on average) than the other treatments (CTR and DIG); at T1-April 2017, amendment increased soil basal respiration according to this trend: CTR < DIG (+141%) < DIGP (+57%), reaching the highest values equal to 837.6 and 1313.9 μg CO$_2$-C g^{-1}(28d)$^{-1}$, respectively for DIG and DIGP; then, a decreasing trend was observed in the other sampling times even though the effect of the amendment (DIG) and its legacy (DIGP), compared to CTR, was always significant and determined higher values by +306% for DIG and +625% for DIGP at T2-June 2017 and +149% for DIG and +289% for DIGP, one year later treatments application, at T3-April 2018 (Trt × T: $P < 0.0001$) (Fig. 3). Solid digestate also augmented soil PMN; after T0, where only the effect of the preceding amendment was observed (+104%) in DIGP, repeated amendment determined the highest PMN concentration at T1-April 2017 (+662% compared to CTR), greater than DIG (+456% compared to CTR); later on, the PMN progressively decreased at T2-June 2017 and one year later, at T3-April 2018, although both digestate treatments (DIG and DIGP) showed higher values than CTR according to the years of application (+224% in T2-June 2017 and +171% in T3-April 2018 for DIG; +300% in T2-June 2017 and +286% in T3-April 2018 for DIGP) (Trt × T: $P < 0.0001$) (Fig. 3).

Among eco-physiological indices, MBC:C_{org} has increased at T1-April 2017 and T2-June 2018 (+76%, on average compared to T0), reaching the highest value equal to 26.3 μg MBC mg^{-1} C_{org} in all plots (with and without digestate); in the last sampling time (one year after treatments application, T3-April 2018) the highest value was found in DIGP

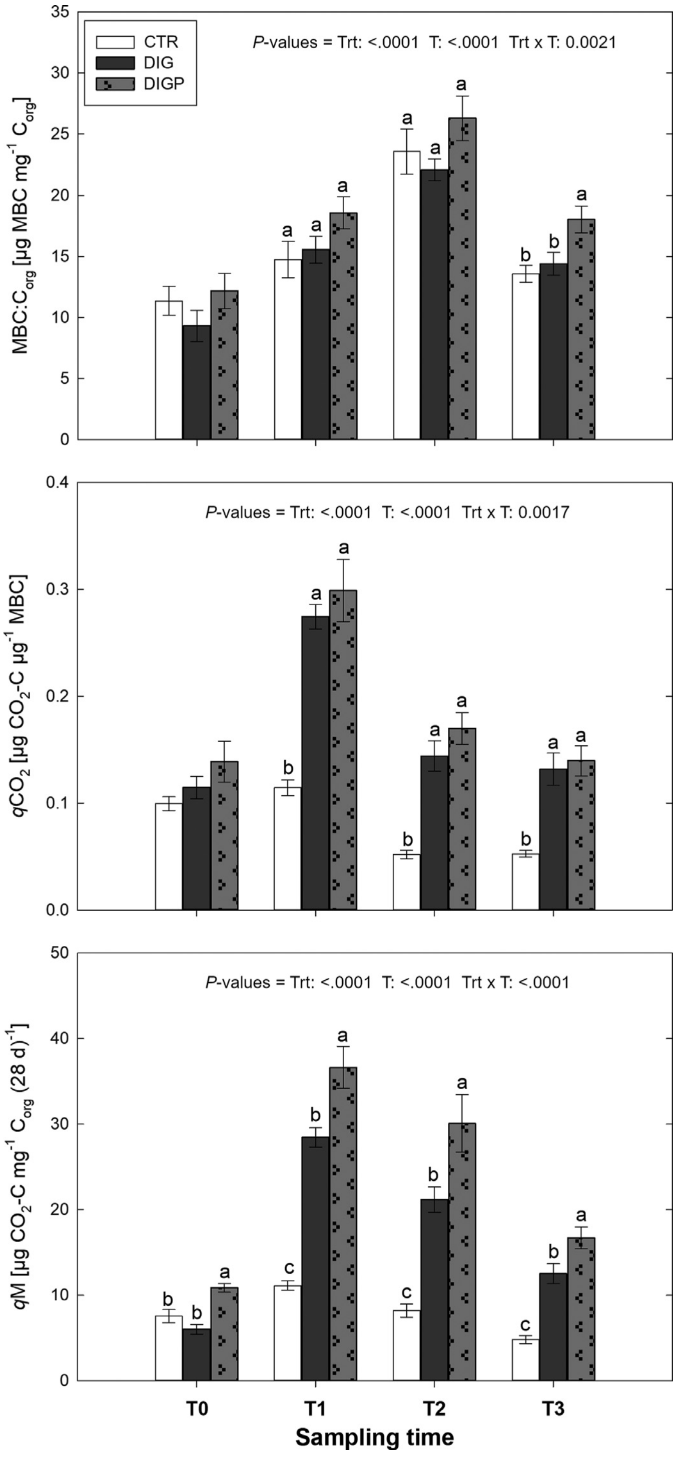

Fig. 4. Soil microbial quotient (MBC:C$_{org}$), metabolic quotient (qCO$_2$) and mineralization coefficient (qM) (mean ± SD, $n = 4$) in the olive orchard soil following the treatments (CTR, DIG, DIGP) at four sampling times (before (T0) and then ~2 weeks (T1), ~3 months (T2) and ~1 year (T3) after the treatment application) during the 2017/2018 cropping season. Within each sampling time, different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < 0.05$).

(+61%) followed by DIG and CTR (Trt × T:$P = 0.0021$) (Fig. 4). Soil qCO$_2$ showed a decreasing trend from T1-April 2017 to T3-April 2018 being higher in digestate amended soils (DIG and DIGP) than under CTR (Trt × T:$P = 0.0017$) (Fig. 4). Similarly, qM showed a decreasing trend among samplings after treatments application from T1-April 2017 to T3-April 2018, with the higher values observed in DIGP followed by DIG, while CTR always showed the lowest values (Trt × T: $P < 0.0001$) (Fig. 4).

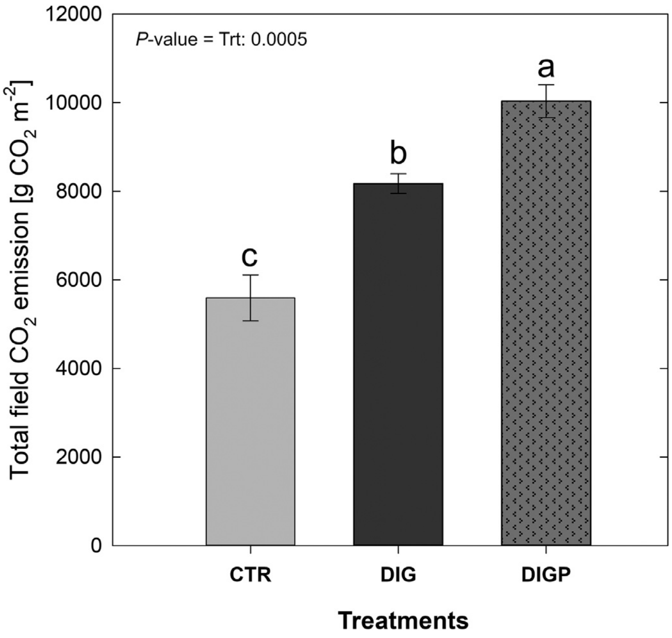

Fig. 5. Total soil CO$_2$ emissions calculated from CO$_2$ emission fluxes monitored in the olive orchard (mean ± SD, $n = 4$) following the treatments (CTR, DIG, DIGP) during 2017/2018 cropping season. Different letters indicate significant differences among soil treatments (Tukey's HSD test at $P < \; < 0.05$).

Trends of soil temperature, water content, CO$_2$ fluxes and total CO$_2$ emission

Soil temperature measured at the 0–5 cm soil layer during the monitoring period (from April 2017 to May 2018) ranged from a minimum value equal to 5 °C, retrieved in January, up to a maximum value of 36 °C reached in July (Fig. S1). The differences among treatments were shorter in the period from spring to summer, while they were higher from autumn to the following spring. Indeed, in this period both amended treatments (DIG and DIGP) showed higher soil temperature values compared to CTR treatment (Fig. S1). Anyway, in the first period after digestate spread (~2.5 weeks), higher soil temperatures were registered (+2.0 °C on average) in both DIG and DIGP treatments. The soil water content (at 0–5 cm soil layer) ranged from 0.09 to 0.51 cm^3 cm^{-3} with slight differences between treatments (Fig. S1). Soil CO$_2$ fluxes during the experimental period ranged from 3.08 to 236.1 g CO$_2$ m^{-2} d^{-1}. Differences among treatments (DIGP > DIG > CTR) were observed from the beginning of the experiment until July, and later, from November until the end of the experiment (May), with major differences among both amended treatments (DIGP and DIG) and the unamended treatment (CTR). Total CO$_2$ soil emission was higher in DIGP (+28%; 10,032 g CO$_2$ m^{-2}) than in DIG (8172 g CO$_2$ m^{-2}), and both were higher than CTR (−69%, on average; equal to 5592 g CO$_2$ m^{-2}) (Trt: $P = 0.0005$) (Fig. 5).

Discussion

Agricultural management and the application of amendments can alter various aspects that are involved in soil functioning with consequences on both the fertility status and CO$_2$ emission. Therefore, the present study aimed to summarize these short-term changes related to single and repeated amendment application evaluating their sustainability for managing soil in olive groves under Mediterranean conditions.

Incorporation of an equal dose of solid anaerobic digestate, either as a single (DIG) or a repeated event (DIGP), determined a significant increase of pH equal to + 0.9. The values reached never exceeded the value of ~6.5, thus remained within the optimum range for nutrient absorption by the plant, improving the original soil pH (5.44), and favouring bacterial cell division and growth (Egene et al., 2021). This evidence agrees with other authors (Hupfauf et al., 2016; Siebielec et al., 2018), who observed how digestate application can increase soil pH because of its alkaline reaction due to the ammonification, $(NH_4)_2CO_3$ formation during the anaerobic digestion (Huang and Chen, 2009; Tambone et al., 2009) and its buffering capacity. Moreover, its effect is more appreciable in acidic soils, like the soil involved in this experiment, that had a greater difference in pH from digestate (Siebielec et al., 2018). The limited and circumscribed effect on pH leads to suppose that constituent soil properties like high clay content and high concentration of Al and Fe, which determine the buffering capacity of the soil, make it not susceptible to a further increase in pH, even when digestate distribution was repeated for two years. So, as argued by Makádi et al. (2012), variations in soil pH due to digestate amendment could appear only after extensive utilization repeated over the long-term.

Incorporating solid anaerobic digestate significantly raised EC, in proportion to the years of use, as an effect of the addition of a high salt content matrix as also reported by Barłóg et al. (2020) and Valentinuzzi et al. (2020). In addition, in digestate amended soils (DIG and DIGP), the increase of soil microbial activity (confirmed by MBC and R_{bas}), consuming organic acids and various compounds, may have contributed to increased EC. The rise of soil EC is one of the most important factors that can limit digestate use, particularly in the long-term repeated use, with the risk to overcome the critical threshold of 2 dS m^{-1} affecting plant growth and crop yield (Almeida Machado and Serralheiro, 2017). However, one year later application, no difference among treatments was observed. Variations among EC values one year after digestate spread (DIG at T0 vs DIG and DIGP at T3) can be attributed to the dissimilar total rainfall in the two cropping cycles (902 mm vs 1031 mm; ARPACAL, 2018) which controls the removal of soluble salts from the soil causing their distribution along the soil profile. Therefore, our evidence suggests that in this agroecosystem, the rainfall regime and soil characteristics permit the annual application of the tested dose of solid anaerobic digestate (30 Mg ha^{-1}) without incurring the risk of soil salinization, but the possible progressive increase in salinity must be monitored over long periods of application.

In our experiment, as reported by several authors (e.g.: Cardelli et al., 2018; Egene et al., 2021), digestate increased organic matter concentration (evidenced by the augmented levels of C_{org} and TN) with a greater effect when the application was performed for two consecutive years (DIGP). This finding, confirms the beneficial effect of solid digestate in increasing the soil organic pools. Incorporation of anaerobic digestate in fine-textured soils, such as that of our experimental site, does not act as a priming factor for mineralisation which can depress the C sequestered in the soil, even when repeated year after year as a regular agricultural practice. It is reasonable to suppose that the interaction between the digestate and the clayey soil promotes C sequestration by physical protectioning the organic matter from microbial degradation, as stated by Churchman et al. (2020). Finally, regarding C sequestration, it is important to highlight the role that digestate amendment can play in protecting or increasing the C stock of olive orchards that represent one of the most important C sinks of the region (Badagliacca et al., 2020b).

Using solid digestate, regardless of the number of applications, suddenly increased N pools (organic and inorganic), as observed in other studies (Pantelopoulos et al., 2016; Cucina et al., 2018), with a lasting effect observable until the final sampling, one year later. The highest mineral N (NH_4^+-N + NO_3^--N) soil concentration, reached in the DIGP treatment, was similar to thet ordinarily supplied by chemical fertilisers in the olive orchards of the region, thus suggesting the use of digestate as an alternative to mineral fertiliser as postulated by Guilayn et al. (2019) and Tsachidou et al. (2019). Moreover, for both amended treatments (DIG and DIGP), mineral N concentration trends along the sampling times suggest that N transformation (mineralisation chain) permits a favourable and continuous N supply to the crop. This occurred by the conjunction between the mineralisation susceptibility of the organic matrix applied (related to its C/N ratio equal to 24) and the soil properties such as the fine texture (clay, with a clay content of 45.0%) and high CEC (51.9 $cmol_+$ kg^{-1}) of the experimental site that protected and modulated organic N (EON) mineralisation and NH_4^+-N nitrification along the time. In any case, it is significant to notice that TSN and mineral N concentration roughly followed the EC pattern. Therefore, considering the option to repeat digestate amendment for consecutive years, according to Insam et al. (2015), it is important to consider the negative effects that may result from high N loads releasing into the soil that could lead to aggregate stability loss and reduction of porosity due to soil sorption complex overload. This risk should suggest to farmers, firstly, to opt for the more stable and time-released nutrients by-products from the anaerobic digestion such as the solid fraction rather than the whole raw digestate or its liquid fraction, and secondly, to take care to monitor the EC over time. With regard to the CTR treatment, it promoted a small transient increase of EON and TSN related to the release of nutrients from the mineralisation of the plant material deposited and available organic matter into the soil, as confirmed by the rise in MBC, R_{bas}, MBC:C_{org} and qM indices.

Amendment with digestate for one year (DIG) and two years (DIGP) increased soil microbial biomass (increasing either MBC and MBN, according to Barra Caracciolo et al. 2015; Cardelli et al. 2018) and its activity (increase of all soil microbial functioning indices, as retrieved by Hupfauf et al., 2016; Cucina et al., 2018), with proportional effects to the number of application years, following the availability of substrates (R_{bas}, mineral N and PMN) and affecting C and N transformation, release or immobilization (Alburquerque et al., 2012; Johansen et al., 2013). Furthermore amendment reveals how the increased availability of readily degradable organic compounds and soil aeration, which improves soil environmental conditions, can promote a long-lasting and stable microbial growth throughout the year, in accordance with Montiel-Rozas et al. (2018) and Lourenço et al. (2020), regardless of the number of years of application.

Assuming the indications provided by the metabolic activity indices (MBC:C_{org}, qCO_2 and qM) it is possible to better understand and formulate hypotheses on the microbial community dynamics in relation to the mineralisation of organic matter under amended conditions. In particular, the microbial quotient (MBC:C_{org}), although it seems to be slightly affected by C and N availability (especially at T3-April 2018), showed similar values among unamended control (CTR) and amended (DIG and DIGP) treatments. Further, in the same way, DIG and DIGP showed no significant qCO_2 differences over the sampling times. Both these results suggest that soil microbial growth in our experimental condition is proportional to the availability of organic substrates and their use efficiency is the same in the amendment treatments (DIG and DIGP), remaining unchanged during the two years of application, despite a greater quantity of readily mineralisable substrates being made available to microorganisms in DIGP. Among the tested treatments, amended (DIG and DIGP) compared to the unamended plots (CTR) showed a sudden and strong increase of qCO_2 values. According to Anderson and Domsch (2010), qCO_2 shows, based on Odum's theory (Odum, 1969), the maintenance carbon demand of the microbial community reflecting microbial enzyme production and microbial biomass turnover rates and the increase in the values of this parameter does not necessarily indicate a stress condition but only a response of the soil biome to short-term environmental change (Zheng et al., 2019). In this regard, in our experiment, the transient increase of this parameter at T1-April 2017 confirmed this condition, while later, at T2-June 2017 and T3-April 2018, the values returned to the same level observed at the beginning of the experiment. The rapid increase of MBC and qCO_2 leads to suppose a dominance of fast-growing r-strategist microorganisms immediately after amendment (rapid response to substrate addition) characterised by low growth efficiency and higher maintenance respiration with a consequently lower C-use efficiency, as postulated by Blagodatskaya et al. (2014) and Geyer et al. (2016). The same cause (higher C and N substrate availability that stimulated R_{bas}) determined higher qM values in both amended treatments (DIG and DIGP). Further, among DIG and DIGP although higher qM values were observed in the two-year (DIGP) than in the single-year (DIG) use, despite the significant R_{bas} increase in DIGP (+64%, on average), the increment was relatively limited (+34%, on average). This evidence together with the $q\mathrm{CO_2}$ trend and C_{org} values leads us to suppose that the repeated application of digestate (with specific characteristics) cannot cause a C degradation spiral in the soil and, on the contrary, allows year after year to increase its sequestered fraction.

In concordance with other experiments (Ren et al., 2017; Verdi et al., 2018; Ray et al., 2020) amendment with organic matrices increased field CO_2 emission. So, our field measurements confirmed the evidence from the incubation in the laboratory (R_{bas}). Digestate amendment producing the concomitant increase of C and N availability (as revealed by R_{bas} and TSN), that feed soil microbiota (confirmed by the increase of MBC and MBN), leads to higher CO_2 emission in the field. In particular, with this regard, the sudden increase of field respiration determined by amendment suggests that the applied matrix, despite the anaerobic digestion process, still contains a relevant amount of easily degradable C substrates, as argued by Pezzolla et al. (2013) and observed by Askri et al. (2016) and Grigatti et al. (2020) in an incubation experiment and from Pampillón-González et al. (2017) in a greenhouse experiment. Among the substrates, carbohydrates, proteins and short-chain organic acids can be easily degraded, especially by r-strategist microorganisms, determining a rapid soil CO_2 flux increase, copying MBC and $q\mathrm{CO_2}$ values, as argued above. After an initial favourable phase for microbial activity, from the beginning of June a reduction in the soil water content may have limited microbial activity; subsequently, in the autumn the respiration rises again and it may have been controlled by microbial biomass turnover and more recalcitrant compounds (hemicellulose, cellulose and lignin) decomposition under aerobic condition (residues of the anaerobic digestion process), as suggested by Cavalli et al. (2017) and Egene et al. (2021). Moreover, the legacy effect of the preceding amendment, influencing the chemical and biochemical parameters of the soil, has as a result increased CO_2 emissions in the DIGP treatment. Furthermore, as postulated by Zimmerman et al. (2011), our results suggest that the microbial community was “got ready” from the preceding digestate application to mineralise available C substrates derived from the second application leading to higher field CO_2 emission. It is important to highlight the role of tillage of the unamended control (CTR) within amended (DIG and DIGP) treatments. Total CO_2 emission in CTR, which can represent the basal emission level due to the soil tillage, accounted for 67.3% of DIG and 52.7% of DIGP total CO_2 emission. Therefore, it is possible to suppose that the emission exceeding that related to CTR (32.7% for DIG and 47.3% for DIGP) can be largely compensated by the benefits for soil fertility and nutrient release for plants derived from the amendment, as revealed by the improvement of all chemical and biochemical indicator investigated in our experiment. In this regard, the augmented soil CO_2 emission may not have an exclusively negative meaning when it is linked to an increase in microbial activity and related processes (e.g. enzymatic) that can be beneficial to soil fertility. Further, calculated C use efficiency (CUE), following the equation proposed by Tiemann and Billings (2011) based on the variation of the MBC and field CO_2 emission between sampling times, showed similar mean values among the two amended treatments (0.15 *vs* 0.14, respectively for DIG and DIGP) confirming, also in the field, the similar C efficiency of single and repeated solid digestate application as retrieved by $q\mathrm{CO_2}$ in the lab and discussed above. The evidence gathered suggest that higher field CO_2 fluxes from amended treatments, and particularly from DIGP, may not determine a soil C over-exploitation and, on the contrary, C sequestration can occur because of the more recalcitrant organic substance (complex lignocellulosic constituents) contained in the matrix. Overall, the present experiment demonstrated that the repeated application of solid digestate (DIGP) permit to improve several soil chemical and biochemical variables related to soil quality with higher benefits compared to the single application (DIG). In particular, DIGP, compared to DIG, allowed to further increase C and N pools in soil, useful for microbial activity, carbon sequestration and plant nutrition, with a slightly higher marginal risk related to soil salinization but highlighting the same utilization efficiency of the supplied organic matter.

Conclusions

Sustainable management of tree crops within the Mediterranean basin, such as olive groves, is of crucial importance to protect soil fertility from degradation and to address current and future agricultural challenges related to increasing agro-ecosystem resilience to climate change. Moreover, the increase in soil-related ecosystem support and regulation services is undoubtedly linked to improvements in crop management techniques that limit agricultural disservices such as erosion, organic matter depletion, loss of biodiversity and pollution. With this aim, the use of organic amendments is one of the main recommended soil management strategies to counteract fertility loss. The findings of our study performed in an olive orchard with clay soil under a Mediterranean environment showed that incorporating solid digestate determined a significant improvement of organic pools providing a long-lasting release of C and N compounds useful to sustain microbial growth and plant nutrition and not showing deleterious effects even in the repeated application. Further, monitoring the soil CO_2 emission along an entire cropping season together with soil properties highlighted that amendment with digestate (in both DIG and DIGP) can be a sustainable management strategy for olive orchard agroecosystems due to its positive effects on all soil chemical and biochemical parameters investigated that can compensate the transient and relatively higher CO_2 emissions. The evidence gathered proved that two consecutively repeated use of solid anaerobic digestate does not have a negative effect on soil fertility and does not cause C over-exploitation, while, on the contrary, can provide increasing benefits to soil fertility and improve sequestered C, despite the higher field CO_2 emission compared to the single application. Overall, this study has highlighted the benefits of solid anaerobic digestate application for the sustainable intensification of olive orchards under the Mediterranean environment, by improving integral soil fertility, in a circular economy scenario capable of valorising agricultural and agroenergy by-products. Further studies on soil properties and abundance and structure of microbial communities are necessary to better understand the mechanism related to mineralisation and nutrient release from the long-term use of solid digestate in Mediterranean soils.

Fig. S1. Soil temperature, volumetric water content and CO_2 emission fluxes (mean, $n = 4$) in the olive orchard soil following the treatments (CTR, DIG, DIGP) during the 2017/2018 cropping season.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Giuseppe Badagliacca: Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Maurizio Romeo:** Methodology, Investigation. **Antonio Gelsomino:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition, Project administration. **Michele Monti:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Acknowledgments

We thank Antonino Zumbo and Clemente Maesano for technical support. This scientific activity 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 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).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.clcb.2022.100004.

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