

Effects of nitrogen fertilizer sources and temperature on soil CO₂ efflux in Italian ryegrass crop under Mediterranean conditions

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Abstract

We report the results of a study that aimed to assess the dynamics of total and heterotrophic soil respiration and its relationships with soil temperature or soil moisture of an Italian ryegrass haycrop managed with different nitrogen (N) fertilizer sources. The field experiment was carried out in the Nitrate Vulnerable Zone of the dairy district of Arborea, a reclaimed wetland in central-western Sardinia, Italy. This is an area characterized by sandy soils, shallow water table and intensive dairy cattle farming systems. Italian ryegrass is grown for hay produc-

tion in the context of a double cropping rotation with silage maize. We analyzed the effects of N fertilizer treatments on soil carbon dioxide (CO₂) efflux, soil water content and soil temperature: i) farmyard manure; ii) cattle slurry; iii) mineral fertilizer; iv) 70 kg ha⁻¹ from slurry and 60 kg ha⁻¹ from mineral fertilizer that corresponds to the prescriptions of the *vulnerable zone management plan*. During the monitoring period, soil water content never fell below 8.6% vol., corresponding to approximately -33 kPa matric potential. Total and heterotrophic soil respiration dynamics were both influenced by soil temperature over winter and early spring, reaching a maximum in the first ten days of April in *manure* and *slurry* treatments. In the last 30 days of the Italian ryegrass crop cycle, total soil respiration decreased and seemed not to be affected by temperature. The analysis of covariance with soil temperature as covariate showed that average respiration rates were significantly higher under the manure treatment and lower with mineral fertilizer than the *slurry* and *slurry+mineral* treatments, but with similar rates of respiration per unit increase of soil temperature for all treatments. The average soil respiration rates were significantly and positively related to the soil carbon (C) inputs derived from fertilizers and preceding crop residuals. We concluded that: i) the fertilizer source influenced soil CO₂ efflux of the winter haycrop according to the amount of C input; and ii) that the temporal dynamics of soil respiration can be explained by soil temperature regime only in winter and early spring. These findings suggest that further studies are needed to analyze the role of soil biological factors controlling soil respiration dynamics of intensive forage cropping systems under Mediterranean conditions.

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Introduction

Soil carbon dioxide (CO₂) efflux, or soil respiration rate (SR), is considered the second largest carbon source in the atmosphere (Schlesinger and Andrews, 2000). The assessment of CO₂ efflux rates can also support the identification of options for cropping systems able to increase soil carbon (C) sequestration. Soil respiration, in particular its heterotrophic component, is strongly related to soil organic carbon (SOC) decomposition (Hanson *et al.*, 2000) and is influenced by the input of organic C into the soil from crop residuals and organic fertilizers (Fliessbach *et al.*, 2007). The application of various organic fertilizers, such as cattle slurry, farmyard manure and crop residuals, was found to influence the soil carbon cycle in intensive maize-based forage systems (Bertora *et al.*, 2009; Tomasoni *et al.*, 2011).

The research findings on the influence of soil temperature (T) and soil water content (SWC) on the soil respiration seasonal dynamics are often contradictory or uncertain, particularly under Mediterranean conditions. In fact, Davidson *et al.* (1998) and Fang and Moncrieff (2001), from laboratory experiments, showed that the seasonal SR

dynamics were positively correlated with T and SWC. In a Mediterranean forestry system of central Italy, Rey *et al.* (2002) observed that T and SR were not correlated in the dry season but there was, however, a positive and linear correlation between SWC and SR. In an irrigated organic cropping system under Mediterranean conditions, Mancinelli *et al.* (2010) also observed a weak correlation between T and SR ($R^2 \leq 0.25$).

Italian ryegrass is widely used as a grass ley in intensive irrigated double cropping systems with silage or grain maize for dairy livestock feeding. In the north-west of Italy, the cultivation of ryegrass in these cropping systems was found to effectively contribute to up to 40% reduction of nitrate leaching relative to the maize monoculture (Zavattaro *et al.*, 2012). In these intensively managed agricultural districts, slurry and/or farmyard manure are a major source of nitrogen (N) for the forage cropping systems, but their use is regulated by local action plans that comply with the EU nitrates (91/676/EC) and water framework (2000/60/EC) directives.

The CO₂ soil efflux was found to be suppressed by mineral N addition (Min *et al.*, 2011) and its seasonal dynamics are affected by mineral N fertilizer rate (Ding *et al.*, 2007). The SR rates are closely related to soil β-glucosidase activity (Mariscal-Sancho *et al.*, 2010), the seasonal dynamics of which are affected by the N fertilizer source (Piotrowska and Koper, 2010). Our hypothesis was that different options for N fertilization management can have an impact on soil CO₂ efflux dynamics during winter and spring, and that such information is relevant to support suitable strategies for an efficient N management for winter forage cropping systems in nitrate vulnerable zones (NVZ) under Mediterranean conditions.

The specific objectives of the present study were: i) to compare the seasonal dynamics of SR and Rh in relation to different N fertilizer sources applied to Italian ryegrass haycrop under Mediterranean irrigated conditions; and ii) to understand how these seasonal dynamics are influenced by SWC or T in relation to the different types of N fertilization.

Materials and methods

Experimental design and management

The field experiment was conducted on a private farm located in a Nitrate Vulnerable Zone in the dairy district of Arborea, Italy (39°47' N 8°33' E, 3 m asl). In this area the main cropping system is a double cropping silage maize – Italian ryegrass rotation. The mean annual temperature and precipitation are approximately 17°C and 600 mm, respectively. The soils were classified as Psammentic Palexeralfs (USDA, 2006). In the top 20 cm, soils had sandy texture (94% sand), bulk density 1.5 g cm⁻³, organic C content 1.4%, C/N ratio 10 and pH 6.3. Olsen P was 70 mg kg⁻¹ and, therefore, optimal for crop growth. According to the estimates with the SPAW Hydrology model (Saxton and Rawls, 2006), the soil water content corresponding to 0, -33 and -1500 kPa were 47%, 8% and 4%, respectively. The average field capacity measured in the field was as high as 20% vol. corresponding to an estimated matric potential of about -23 kPa and to a hydraulic conductivity of less than 0.002 mm h⁻¹ (De Sanctis *et al.*, 2011).

Four fertilizer sources were compared at the same level of N target rate (130 kg ha⁻¹), set on the basis of the N fertilization prescriptions for nitrate vulnerable zones and on the crop N requirements (Table 1). These were: i) *manure* (mature cattle manure applied before sowing with a conventional spreader and followed by rotary tillage); ii) *slurry* (cattle slurry applied before sowing with a conventional spreader and followed by rotary tillage); iii) *mineral* (mineral fertilizer (ENTECH 26[®]) applied at the end of tillering); iv) control (*slurry+mineral*, i.e. slurry as above but at a target rate of 70 kg ha⁻¹ N and mineral fertilizer (ENTECH

Table 1. Agronomic management practices applied to the Italian ryegrass crop.

| Management practices | Date |
|-------------------------------------|--|
| Organic fertilization | 20 th October 2010 |
| Harrowing (30 cm depth) and seeding | 21 st October 2010 |
| Mineral fertilization ^o | 8 th March 2011 |
| Sprinkler irrigation (4h×5.4 mm/h) | 8 th April 2011 [#] 15 th April 2011 |
| Hay mowing | 16 th May 2011 |

^oMineral and slurry+mineral treatments; [#]irrigation was applied soon after soil respiration measurements.

26[®]) at a rate of 60 kg ha⁻¹ N applied at the end of ryegrass tillering). The experimental design was a 4×4 latin square design with a plot size of 12×60 m².

A mixture of four varieties and hybrids of Italian ryegrass (*Lolium multiflorum* Lam. cv Meritra, Ivan, Littorio and Mowester) was sown over the last ten days of October 2010 following organic fertilizer applications, except for the mineral treatment, and disc harrowing (Table 1). In April 2011, auxiliary irrigation (43 mm split in two applications) was provided when necessary to minimize crop water stress. Hay was harvested in mid-May.

Measurements

Soil respiration efflux was measured *in situ* using a portable, closed chamber, soil respiration system (EGM-4 with SRC-1, PP-Systems, Hitchin, UK) with a measurement time of 120 s. After seeding, three PVC collars per plot (10 cm inner diameter and 10 cm long, with perforated walls in the first 5 cm) were inserted into the soil to a depth of 9 cm. During each CO₂ efflux measurement, the SRC-1 chamber was fitted to a collar. In order to estimate heterotrophic respiration, at each plot one of the three collars was placed on a root exclusion subplot where soil was isolated with a PVC cylinder (40 cm diameter, 40 cm high) opened at both ends, following the method described by Alberti *et al.* (2010). The two SR measurements per plot were pooled together. Therefore, at each date of sampling, one average SR and one Rh value per plot (16 values for each date) were used for data analysis. Soil respiration was measured nine times from January to May 2011 at a frequency depending on the weather variability and agricultural practices. Soil respiration was always measured between 8:30 and 12:00 am standard time, according to Xu and Qi (2001). Soil T and SWC were measured at each plot at the same time of CO₂ efflux measurement using, respectively, a digital thermometer HD2101.2 (Delta Ohm, Padova, Italy) and the Diviner 2000 (Sentek, Stepney, Australia), an instrument based on Frequency Domain Reflectometry. SWC and T were measured, respectively, in the top 20 cm of soil and at a depth of 10 cm in order to analyze their relationships with SR and Rh (Davidson *et al.*, 1998).

The relationship between SR and T was analyzed according to the model: $SR = a e^{bT}$ (Davidson *et al.*, 1998), where T is the soil temperature at 10 cm depth and a and b are the equation parameters.

The C inputs to the soil were estimated from the C left in the soil by the previous maize crop assuming a mean C content of maize dry matter residuals of 0.44% (Bertora *et al.*, 2009) and on the basis of the measured maize yields (*data not shown*), plus the C inputs deriving from the organic fertilizers. The maize crop residuals were estimated to be 18% of the harvested biomass. The hay production was determined by weighing the hay round bales obtained at each plot. The C content of organic fertilizers was determined by the Springer-Kleen method (Mipaaf, 2006).

Statistical analysis

Temperature, SWC, SR and Rh data were analyzed according to an analysis of variance with repeated measures over time using general linear models in the SAS statistical software (proc MIXED, repeated option) (SAS, 1999). Type of N fertilizers and dates of measurements represented the treatments. SR and Rh variables were transformed into log values prior to submitting them to the analysis of variance, to meet the homogeneity of error variance assumption (Gomez and Gomez, 1984). Treatment means were compared using a protected least significant difference test at $P < 0.05$.

The significance of the linear regressions between soil T and the log-transformed SR or Rh rates was tested by checking for discontinuity of the relationship with time through a step-by-step procedure, *i.e.* by removing one measurement date at a time. When the relationships between soil T and SR or Rh was significant, the analysis of covariance was performed on log-transformed values of SR and Rh to check the effect of fertilizer type independently of the influence of soil T (covariate).

Finally, the linear regression analysis was performed to test the relationships between soil C input and SR or Rh rates, averaged over the whole observation period.

Results

There was a wide variation in both SWC and soil T through the observation period ($P < 0.001$). Maximum SWC coincided with the lowest soil T in January, while maximum soil T values were recorded in May when SWC showed the lowest values (Figure 1A). During the observation period, SWC never fell below 8.6% vol. corresponding to approximately -33kPa matric potential (Saxton and Rawls, 2006). SWC was not significantly influenced by treatment type, apart from significantly lower values ($P < 0.05$) for the mineral fertilizer on the 8th of April. Soil T was significantly lower under the *mineral* fertilization treatment than under the *manure* and the *slurry* treatments on the 1st of March and on all the measurement dates in April and May.

SR varied markedly over the period of observation showing a decreasing trend after the maximum observed on the 8th of April (Figure 1B). SR was significantly lower in the mineral treatment than in the two organic fertilizer treatments on the 14th and 21st of March, *i.e.* one and two weeks after the mineral fertilizer distribution, respectively. Regarding Rh, the most marked differences between treatments were observed on the 8th of April with both organic fertilizer treatments showing higher values than the *mineral* or *mineral+slurry* treatments. On average, SR or Rh levels were lower in the *mineral* treatment than in the other treatments including organic fertilizers (Figure 1B and C). SR and Rh were significantly correlated in the January-April period ($r = 0.84^{**}$, $n = 24$), while no significant correlation between the two variables was found in May (*data not shown*). In the same period, treatment type did not influence the rates of increase of SR or Rh with soil T. The dynamics of SR or Rh were positively influenced by soil T only in the January-April period (Figure 2). In May, soil T was negatively correlated with SR while it did not significantly influence Rh. No significant relationships were observed between SWC and SR or Rh (*data not shown*), irrespectively of the period and the type of N fertilizer.

The hay dry matter yield was significantly higher in the *mineral* and in the *slurry+mineral* treatments than in the two organic fertilizer ones (5.4 *vs.* 2.4 t ha⁻¹, respectively). There was no significant difference in hay yield between *manure* and *slurry* and between the *mineral* and the *slurry+mineral* treatments. The SR and Rh values averaged over the nine dates of observation significantly increased linearly with the amount of C input deriving from the fertilizers and from the preceding crop (maize) residuals at a rate of 5.8 and 4.6 g d⁻¹ C efflux kg⁻¹ input C, respectively (Figure 3). The contribution to the total C input from the residuals of the preceding crop corresponded to 36%, 51%, 68% and 100% in the *manure*, *slurry*, *slurry+mineral* and *mineral* treatments, respectively (Table 2).

Table 2. Carbon input in relation to the nitrogen fertilizer source.

| Treatments | Carbon input from fertilizer (kg ha ⁻¹) | Estimated carbon input from maize crop residuals (kg ha ⁻¹) | Total carbon input (kg ha ⁻¹) |
|----------------|---|---|---|
| Manure | 2896 | 1647 | 4543 |
| Slurry | 1656 | 1764 | 3420 |
| Slurry+mineral | 889 | 1862 | 2751 |
| Mineral | 0 | 1852 | 1852 |

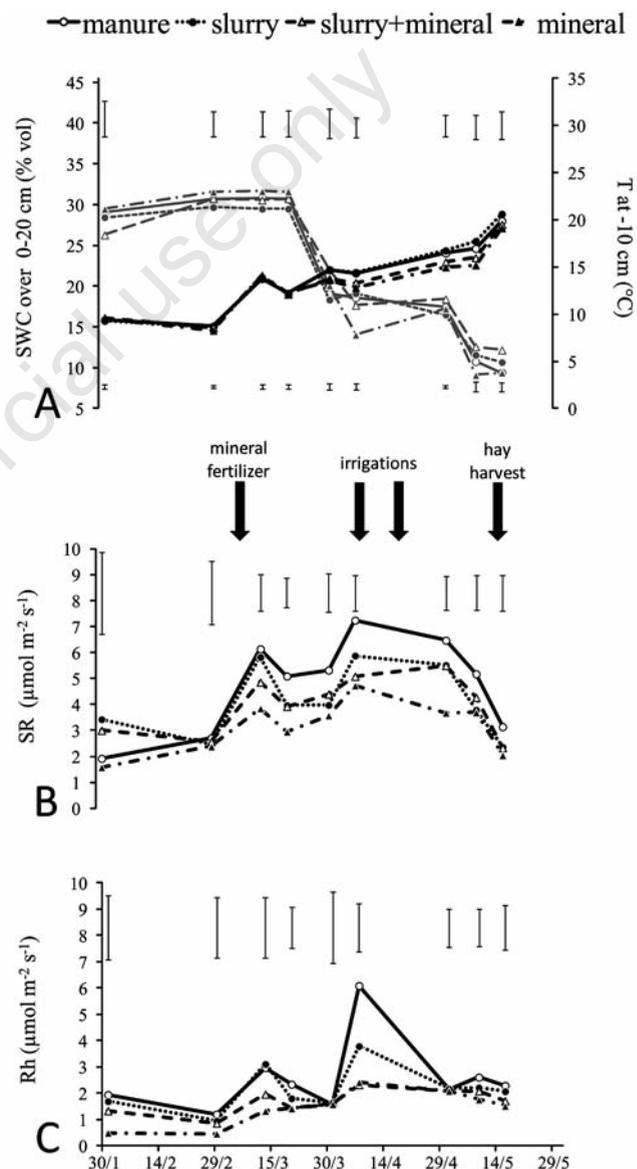


Figure 1. Seasonal dynamics of soil water content (A, gray lines), soil T (A, black lines), soil total respiration (B) and soil heterotrophic respiration (C) observed during the monitoring period in relation to the nitrogen fertilizer sources. Bars represent least significant differences for $P < 0.05$ on each measurement date.

Discussion

The observed dynamics of SR, Rh, SWC and T confirmed those reported in other studies under Mediterranean conditions (Ray *et al.*, 2002; Almagro *et al.*, 2009; Cotrufo *et al.*, 2011).

The lower T values in spring of the *mineral* treatment in comparison with the others were mainly attributed to a higher plant cover and sward height, which also corresponded to lower SWC. Furthermore, lower T values under mineral *versus* organic fertilization in spring were consistent with the observed lower SR and Rh which were likely associated to a reduced microbial activity due to a lower organic C input.

The lack of significant relationship between observed SWC and soil respiration observed is consistent with the findings of Davidson *et al.* (1998), Rey *et al.* (2002), Almagro *et al.* (2009) and Oyonarte *et al.* (2012) who reported significant effects of soil moisture at SWC values far lower than the upper drainage limit (-33kPa). In our experimental conditions, SWC showed a relatively wide variation along the monitoring period, but never fell below a corresponding matric potential of -33kPa, as expected for an irrigated haycrop. Consequently, soil T was found to be the most relevant factor affecting soil CO₂ efflux dynamics within treatment, but only in the winter and early spring. The negative correlation between T and SR observed in May was found also by Oyonarte *et al.* (2012) and was attributed to a decrease in root respiration due to gradual crop senescence. The absence of relationships between T and Rh in May might be attributed to the time dynamics of the site-specific bacterial community composition driving the soil C and N mineralization processes (Wheatley *et al.*, 2003). These findings partially confirmed reports by Rey *et al.* (2011) who found an alternate

effect of T or SWC on the seasonal soil respiration dynamics. These different trends show that under Mediterranean conditions, soil respiration can hardly be simulated by models based only on SWC and T dynamics, particularly in spring, also when continuous automated systems for data collections are used (Cotrufo *et al.*, 2011).

The very low crop production obtained following the *manure* and *slurry* applications was interpreted as an outcome of a severe N stress in late winter and early spring. This was attributed to a low soil mineral N content after autumn and to the early winter nitrate leaching associated to the water surplus (*data not shown*) and to the imbalance between N crop requirements *versus* soil organic N mineralization rates, of which CO₂ efflux can be considered a proxy. However, the response to soil T was relatively flat in the range of values observed from January to May (8-13°C). From mid-March, the significantly lower SR or Rh rates observed for the *mineral* treatment is consistent with the mineral N inhibition on the microbial communities implicated in SOC decomposition (Lagomarsino *et al.*, 2007).

The inhibition of SR or Rh following the addition of *mineral* N did not influence the rate of increase of SR or Rh *versus* T rise. Furthermore, we did not find any significant differences in terms of the differential response of SR to T rise between *manure* and *slurry* treatments in winter and early spring, despite the different chemical composition of the two components (Bertora *et al.*, 2009) and the different kinetic properties of various organic matter inputs (Davidson and Janssens, 2006). The interpretation of these results would have required additional field data on soil microbial community and SOC dynamics during the experimental period, and should be the subject of further investigation. We hypothesized that the steady rate of increase of SR or Rh *versus* rise in T could be explained by the resistance of

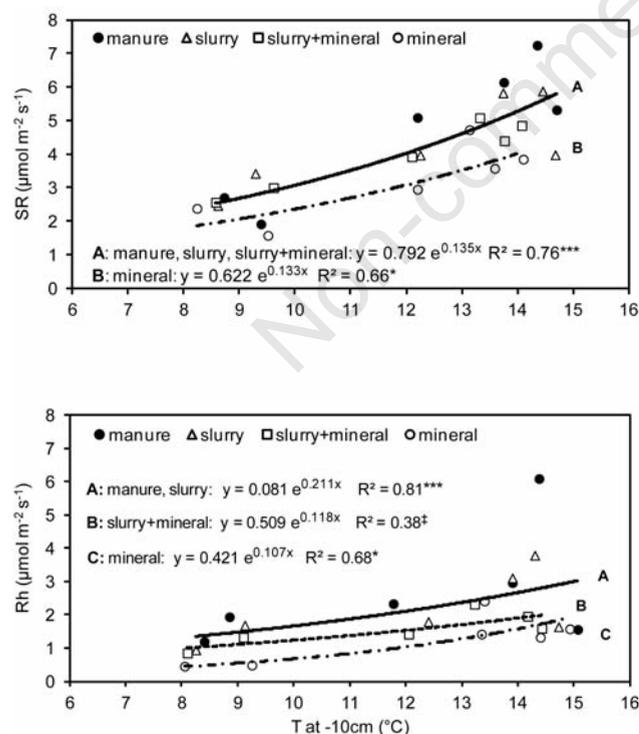


Figure 2. Exponential relationships (SR or $Rh = a e^{bT}$) between temperature (T) at 10 cm soil depth and soil total respiration (SR, top) or soil heterotrophic respiration (Rh, bottom) in the January-April period. $^{***}P < 0.001$; $^*P < 0.05$; $^{\ddagger}P < 0.06$.

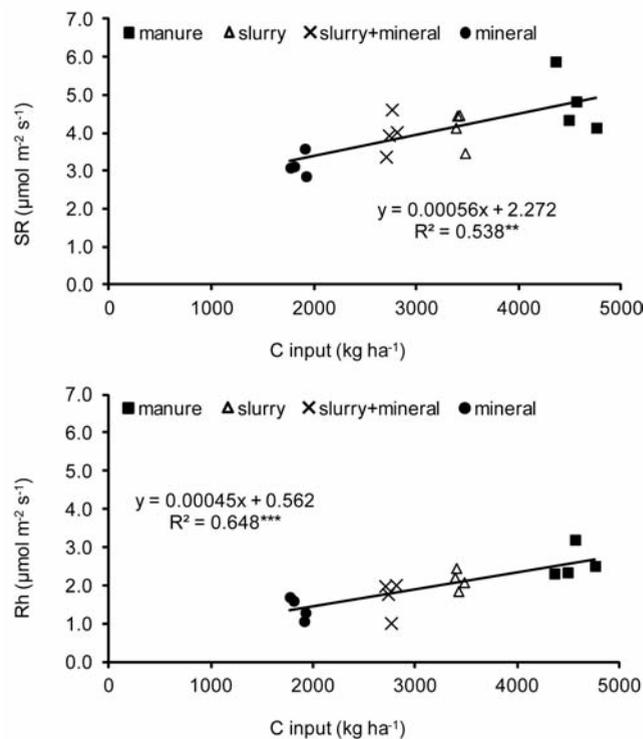


Figure 3. Relationship between soil carbon input and soil total respiration (SR, top) and soil heterotrophic respiration (Rh, bottom) averaged over measurement dates. $^{***}P < 0.001$; $^{**}P < 0.01$.

microbial communities to environmental nutrient changes, as described by Bowen *et al.* (2011). This hypothesis is also consistent with reports by Janssens *et al.* (2010), Lagomarsino *et al.* (2011) and Schmidt *et al.* (2011) who highlighted that the stability of the soil microbial community structure and ecological equilibrium were affected by the specific long-term management history.

The effects of the type of fertilizers on SR or Rh was difficult to detect also because of the high error variance within each measurement date which increased linearly with the amount of C input of the different fertilizers (*manure>slurry>slurry+mineral>mineral*). When considering the means of the single replications over all the sampling dates, such a variability was flattened and a significant relationship emerged between SR or Rh and the amount of C input from the different treatments.

The relatively low soil respiration values observed following the N fertilization in the *mineral* and *slurry+mineral* were associated to the low C input (only residuals from the previous crop) and to the above mentioned inhibitory effect of the high soil N mineral availability for the microbial communities (Lagomarsino *et al.*, 2007).

Conclusions

The effects of the different types of N fertilizers on soil CO₂ efflux can be explained by the combination of soil T, amount of C input and by the inhibitory effect of soil mineral N availability. However, further field measurements are required to confirm the latter hypothesis.

Soil T was a driver of soil respiration only in winter and early spring while after this period, despite further T increase, SR decreased during crop senescence while Rh values were quite stable and low for all treatments, even if SWC was never limiting. The rate of increase of SR or Rh with soil T during winter and early spring was not influenced by the type of fertilizer, although both SR and Rh values were lower at the same T level following the application of mineral N fertilizer.

The amount of C input derived from fertilizers and residuals of the preceding crop was found to be one of the main drivers of the mean SR or Rh, particularly in the second half of the crop cycle. However, the drivers of the seasonal dynamics of CO₂ efflux were not fully understood, and the rates of SR and Rh measured in winter and early spring were not always consistent with an imbalance between organic matter mineralization and crop N uptake, leading to severe N stress of the organic treatments.

Further field experiments are, therefore, recommended to understand the role of soil biological features, such as the composition and functioning of the microbial populations in regulating the soil CO₂ efflux of intensive forage cropping systems grown under Mediterranean conditions.

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