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## The effect of Hydropolymers on soil microbial activities in Mediterranean areas

El efecto de los polímeros absorbentes en la actividad microbiana del suelo bajo condiciones mediterráneas

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### ABSTRACT:

**Objectives.** The aim of this study was to evaluate the effect of the hydropolymer TerraCottem on soil microbial activity by measuring soil respiration and leaching of mineral nitrogen. **Materials and methods.** The incubation experiment contained control variants with natural soil of Nerja area (South Spain, inside the Sierra Tejeda, Almajara and Alhama Natural Park, 36.7985173° N 3.8511693° W; WGS84), variants with the addition of easy available nitrogen compounds (kg N ha<sup>-1</sup>), easy accessible carbon compounds (1% glucose solution) and a combinations of both. Within each variant, the recommended amount of control hydropolymers (1.5 kg/m<sup>3</sup>) and a double dose of 3.0 kg/m<sup>3</sup> were compared. **Results.** Showed that respiration activity of the soil in this Mediterranean area was not eliminated by the lack of ready available nitrogen or carbon substrates. Furthermore, differences in CO<sub>2</sub> production between the variants containing different amounts of hydropolymers were not significant. A statistically significant difference in the CO<sub>2</sub> production was found in the first week compared to longer time periods. **Conclusions.** The mineral nitrogen leaching measurement showed that the biological activity of the studied is not affected by nitrogen dynamics which is balanced regardless of the amount of applied hydropolymer. On the other hand, leaching processes occurred when soil was doped only with nitrogen compounds or only carbonaceous, a problem that can appear after fertilizers applicatio

**KEYWORDS:** soil conditioners, respiration, leaching, terracottem, nitrogen.

### RESUMEN:

**Objetivo.** El objetivo de este estudio pasa por evaluar el efecto del-Terracottem- en la actividad microbiana del suelo mediante la medida de la respiración edáfica y la pérdida de nitrógeno mineral por lixiviación. El ensayo se ha realizado en condiciones de laboratorio controladas y con suelos naturales. **Materiales y métodos.** Para el experimento, se han diseñado varios tratamientos: i) suelos naturales a los que se les ha adicionado compuestos nitrogenados de libre disponibilidad (Kg N ha<sup>-1</sup>); ii) suelos naturales a los que se les ha adicionado carbohidratos de libre disponibilidad (1% de solución de glucosa); iii) suelos naturales a los que se les ha adicionado una mezcla de compuestos nitrogenados de libre disponibilidad (Kg N ha<sup>-1</sup>) con carbohidratos (1% de solución de glucosa). En cada variante se han testeado diferentes dosis del polímero. **Resultados.** Los resultados han demostrado que la actividad respiratoria del suelo es independiente de la disponibilidad de compuestos como el nitrógeno o carbono. Tampoco se han observado diferencias significativas entre las diferentes dosis del polímero. Por el contrario, si se observaron diferencias en la producción de CO<sub>2</sub>. **Conclusiones.** La lixiviación únicamente se producía cuando los suelos era enmendado con compuestos únicamente nitrogenados o únicamente sólo carbonosos.

**PALABRAS CLAVE:** acondicionador de suelos, respiración, lixiviados, Terracottem, nitrógeno.

## INTRODUCTION

Around 80% of ecosystem services can be connected to soil functions. However, these services can be critically altered by the effects of anthropogenic activities (tillage on slopes, deforestation, pasture production, etc.) and desertification processes (1).

Soil parameters are commonly used to estimate soil quality or functionality in restoration including individual determinations of basic physical and chemical factors as the soil organic matter content or soil loss (2); however, these parameters fail to account for biological processes. Therefore, activities of soil microbiota can be used as a significant indicator of the soil quality in degraded areas (3).

During dry periods, in Mediterranean areas, the lack of water entering the soil matrix reduces organic contributions via plant remnants and rhizodepositions to the soil. These processes lead to reduced fertility Commented [MMR3]: and ecological functions of soils (4). The effect of restored vegetation communities can be decisive to regenerate the soil functionality. Thus, the restoration of degraded ecosystems should aim not only to recover the capacity of soil to support vegetation, but also to restore the ecosystem functions and services (5). Sowing, germination and plant establishment stages are critical (6), but during these stages the beneficial effects of the vegetation may not be apparent, and the soil is highly susceptible to erosion and depletion of soil organic carbon (1). Under these conditions, vegetation cover in areas with degraded soils may be better sustained with an external sources of soil organic matter (7).

Synthetic polymers such as hydrogels have proved to be an effective method to promote plant establishment in semiarid areas. These polymers are also called 'water absorbents' because of their high capacity to retain water (8). Terracottem is a kind of hydroabsorbent polymer that consists of a mixture of over twenty components working in synergy. This product was developed in Ghent University (Belgium) to improve plant growth, and especially to enhance the resilience of soil to drought conditions (9). According to the producer, Terracottem significantly increases the capacity of soils and growing media to retain and provide water and nutrients, which promotes the growth of plant roots and shoots. The addition of this material to soils also increases water infiltration rates (1). All of these factors contribute to increasing soil water availability for plant growth and for successful and sustainable land management (10). The main portion of Terracottem is composed of a mix of 19 gel fragments acting as small individual reservoirs of available water for plants, and the remaining 10.5% is composed by a fertilizer (11). Many authors have demonstrated the positive effect of Terracotterm on soil characteristics, e.g. water infiltration or cation exchange capacity (12). Thus, the above mentioned studies have shown that synthetic polymers such as hydrogels could be used as a promising method to restore some Mediterranean degraded soils.

Microbial indicators are more sensitive to disturbances than physical and chemical parameters and should be routinely incorporated in soil assessment studies in restoration programs. Soil microbial activities and diversity play key roles in the sustainability of vegetation communities by maintaining vital functions in the soil part of ecosystem, involving carbon and nutrient cycling (10). Enhancing soil microflora can increase the amount of nutrients available to plants and promote the symbiosis plant/fungus to expand the root surface (11).

The aim of this study was to investigate the effects of Terracottem on soil microbiota in relation to restoration of soil functions and services. The specific objectives of the study were to: 1) analyse the soil respiration in soil amended with different doses of Terracottem; 2) determine the leaching of nitrogen mechanism affected by different doses of Terracottem; and 3) determine the effectiveness of Terracottem to restore the soil function in Mediterranean degraded soils.

## MATERIAL AND METHODS

**Soil characteristics.** Soil characteristics. Soil used in all experiments was obtained from the El Pinarillo experimental site (mountainous region of southern Spain, inside the Sierra Tejada, Almijara and Alhama Natural Park 36.7985173° N 3.8511693° W; WCGS84), in spring 2015 (X: 424,240 m; Y: 4,073,098 m; UTM30N/ED50). According to the Food and Agriculture Organization of the United Nations (FAO) — the World Reference Base for Soil Resources (2006), the soils are classified as lithic and eutric leptosols. They are characterized by a high level of rock fragment cover on the surface (>50%), a high gravel content in the profile (total gravel content =56%; gravel content >10 mm =31%; gravel content 2 fine (f) mm =10%; gravel content 5f 1 mm =15%), and a sandy loam texture (sand =60%, silt =32%, and clay =8%). The general soil properties and characteristics of the soil are given in Table 1.

TABLE 1.  
Table 1. Climatic conditions and soil properties under natural conditions at the El Pinarillo experimental site.

Annual rainfall	(mm/y)	589.0
Climatic regime		Dry
Soil properties		
Gravel	(%)	56.0
Sand	(%)	60.0
Silt	(%)	32.0
Clay	(%)	8.0
Field capacity SWC	(%)	25.2
Wilting point SWC	(%)	6.4
Available water	(%)	18.8
Aggregate stability	(%)	51.0
CEC	Meq/100 g	120.4
TC	(%)	12.5
TN	(%)	0.2
C/N		9.5
SOC	(%)	1.9
pH		8.0
EC	( $\mu$ S/cm)	501.0
CEC: cationic exchangeable capacity, TC: total carbon, TN: total nitrogen, C/N: carbon/nitrogen ratio, EC: electrical conductivity, SOC: organic carbon content, SWC: soil water content.		

Soil, excluding surface litter, was collected randomly (10 samples) at the study site to the depth of 25 cm using a soil sampler, and was combined into one composite sample. The mixed soil sample was taken to the laboratory (Geomorphology and Soil laboratory, University of Málaga, Spain) within 24 hr from the sampling and sieved through a 2 mm mesh, removing visible pebbles and roots. The homogenised soil was left to dry at 23°C in the laboratory and then stored in plastic bags in the refrigerator (at 4°C) and kept there until the start of experiments.

**Experimental design.** The incubation experiment with the homogenised soil was conducted in June 2015 using plastic containers (volume??, n=48). Each plastic container, was filled with 200 g of the soil sample and left incubated under 15% soil moisture. Soil conditions were maintained constant during the incubation

period. All 48 containers were placed in a randomized block design, and during the incubation period they were rotated periodically to minimize any systematic failure.

In July 2015, after one week of the pre-incubation, different amendments of a hydrolymer, glucose, nitrogen fertilizer and their combinations were added to test the activity of soil microorganisms. DAM 390 was used as a nitrogen fertilizer, which is a solution of ammonium nitrate and urea with an average content of 30% nitrogen. The amount of a mineral nitrogen fertilizer corresponds to a dose of 50 kg N ha<sup>-1</sup>. The form of nitrogen application was made as a solution.

The glucose addition was the same as recommended by Ghani et al (12), 1% of dry matter (DM) of soil. These additives were applied at the end of the first week of the incubation. During the remaining three weeks, the soil moisture was regularly controlled and kept at 15% of moisture by the addition of distilled water.

Two doses of hydrolymers TerraCottem Universal were tested (Table 2): i) 1.5 kg m<sup>-3</sup> and ii) 3.0 kg m<sup>-3</sup> during the incubation according to the specification of the producer of TerraCottem. Hydrolymers were thoroughly mixed with the soil before inserting the mixture into the containers. Four containers without any additions were used as the control samples.

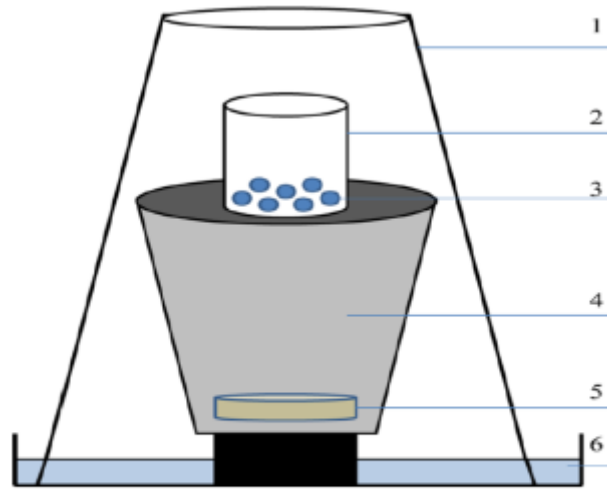
TABLE 2.

Table 2. Setting up of different substrate treatments. 4 repetitions.

Treatment	Terracottem	Additives
A0	-	-
A1	1.5 kg m <sup>-3</sup>	-
A2	3.0 kg m <sup>-3</sup>	-
B0	-	Glucose (1%)
B1	1.5 kg m <sup>-3</sup>	Glucose (1%)
B2	3.0 kg m <sup>-3</sup>	Glucose (1%)
C0	-	Nitrogen fertilizer (50 kg N ha <sup>-1</sup> )
C1	1.5 kg m <sup>-3</sup>	Nitrogen fertilizer (50 kg N ha <sup>-1</sup> )
C2	3.0 kg m <sup>-3</sup>	Nitrogen fertilizer (50 kg N ha <sup>-1</sup> )
D0	-	Glucose (1%) + Nitrogen fertilizer (50 kg N ha <sup>-1</sup> )
D1	1.5 kg m <sup>-3</sup>	Glucose (1%) + Nitrogen fertilizer (50 kg N ha <sup>-1</sup> )
D2	3.0 kg m <sup>-3</sup>	Glucose (1%) + Nitrogen fertilizer (50 kg N ha <sup>-1</sup> )

### Measurements

**Soil respiration.** During four weeks, the soil respiration was measured every seven days with alkali absorption of CO<sub>2</sub> by soda lime according to the methodology proposed by Keith and Wong (13). A plastic container with 12 g of dry soda lime was placed on the surface of the soil sample and airtightly closed with a sealing chamber (Figure 1). After 24 hours, the production of CO<sub>2</sub> was measured.



**Figure 1.** Diagram illustrating the pot with a soil sample, soda lime trap, IER disc, and a sealing chamber design, where: 1- Sealing glass chamber, 2 -Plastic container, 3- Soda Lime, 4 - Container with soil, 5 -IER discs, 6 - Container with distillate water

FIGURE 1  
Figure 1

The production of CO<sub>2</sub> [R; (g C m<sup>-2</sup> IP<sup>-1</sup>); IP ~ incubation period] was expressed according to the formula of Keith & Wong (2006):

$$\left\{ \frac{[(\text{sample weight gain}(g) - \text{mean blank weight gain}(g)) \times 1.69]}{\text{chamber area}(m^2)} \right\} \times \left[ \frac{24(\text{hr})}{\text{duration of exposure}(hr)} \right] \times \left[ \frac{12}{44} \right]$$

To obtain the cumulative production of CO<sub>2</sub> during the whole week before the repeated rewetting, respiration rate on the first day was multiplied by an empirical constant (C=3.5), obtained in preliminary experiments. This reflects the remaining production of CO<sub>2</sub> during next six days, when the alkali trap was not present inside the chamber in order to avoid underestimation of the process of nitrification, which could be limited by the insufficient amount of CO<sub>2</sub> (14).

**Leaching of nitrogen.** Leaching of mineral nitrogen (N<sub>min</sub>), calculated as the sum of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N was assessed by using ion exchange resin (IER) flat covers (bags) similar to those used by Binkley and Matson (15). The IER bags for trapping N<sub>min</sub> were placed in the sand layer at the bottom of the pots under the soil sample, and exposed there for 4 weeks (see Figure 1). N<sub>min</sub> captured into IER was extracted into the solution of 1.7 M NaCl and determined by the distillation-titration method according to Binkley and Matson (15).

**Statistical analysis.** Statistically significant differences were determined using analysis of variance (ANOVA). The assumption of homoscedasticity was tested using Levene's test. In cases of non-homoscedasticity (Levene test; p<0.05), Kruskal-Wallis test was used. Mean differences between the various experimental soil treatments were determined using Tukey's test or the Games-Howell test. In all the

analyses the selected significance limit was  $p < 0.05$ . The analyses were performed using SPSS (version 23) for Windows.

## RESULTS

**Effects of carbon and nitrogen addition on soil respiration.** Soil respiration was increased (a) by the addition of glucose (easily available carbon), (b) after the addition of nitrogen fertilizer, but significantly only (c) after the combined addition of both, glucose together with the nitrogen fertilizer (Figure 2).

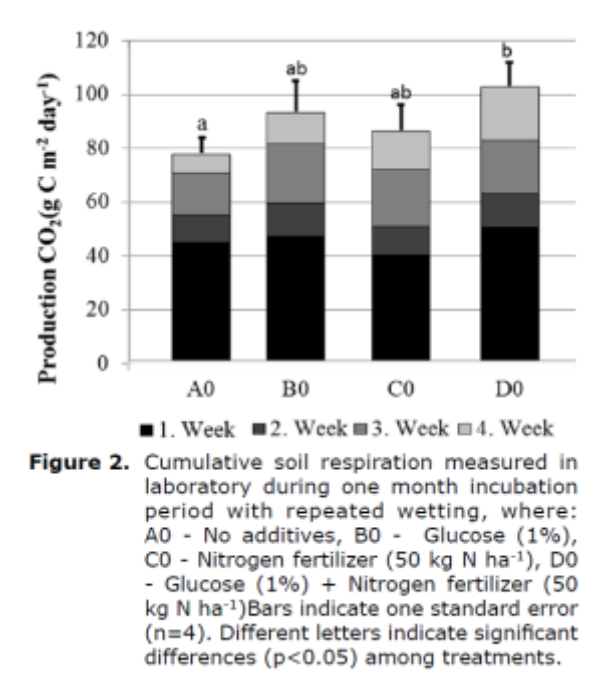
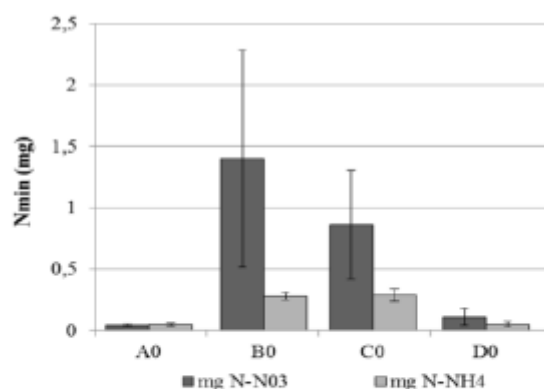


FIGURE 2  
Figure 2

The highest values of respiration were noted during the first week in all treatments. In the A0, B0 and D0 treatments, respiration exceeded the sum of respiration in that time compared to the remaining weeks. This response can be a consequence of the disturbances of the experimental soil samples during soil sampling and homogenization, even if the experimental soil was exposed to the period of one month preincubation to avoid it.

**Leaching of mineral nitrogen.** The values of nitrogen leaching measured in C0 treatment were higher than those found in the control samples (Figure 3). This could be explained by the addition of nitrogen fertilizer to the soil, and insufficient amount of carbon for preventing nitrogen immobilization by microorganisms.



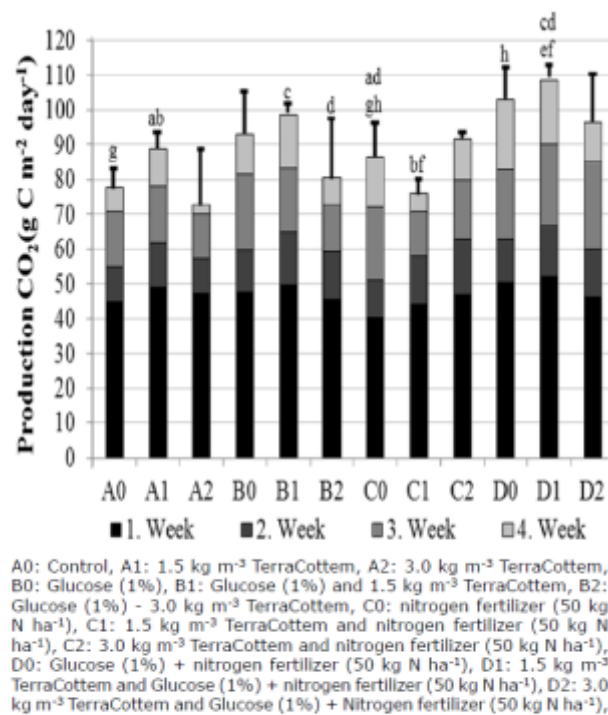
**Figure 3.** Mean and standard deviation (SD±) of mineral nitrogen leaching after the application of 3 types of additives. A0: Control, B0: Glucose (1%), C0: Nitrogen fertilizer (50 kg N ha<sup>-1</sup>), D0: Glucose (1%) + Nitrogen fertilizer (50 kg N ha<sup>-1</sup>), during one month incubation period with repeated wetting.

FIGURE 3

Figure 3

The application of easy available carbon (glucose) in the B0 treatment may have caused faster mineralization of soil organic nitrogen, which led to the greater subsequent leaching of mineral nitrogen. (Figure 3). These results indicate that an extra source of carbon and nitrogen added to the experimental soil can cause the overall support for microbial activity. However, providing only carbon or nitrogen, destabilization in the natural balance of C/N can promote an increase in the nitrogen loss (16) (Tables 2 and 3).

**The effect of TerraCottem on soil respiration.** According Figure 4 the highest production of CO<sub>2</sub> was measured in first week for all rates of polymer and all additives (A1, B1, C1, D1 and A2, B2, C2, D2). TerraCottem doesn't affect soil respiration in the first week.



**Figure 4.** Cumulative CO<sub>2</sub> production (soil respiration) measured in the laboratory during one month incubation period with repeated wetting after applying of TerraCotem and additives together.

**The effect of TerraCottem on nitrogen leaching.** Differences in nitrogen leaching among TerraCottem treatments, disregarding of the doses used, were not significant (Table 3 and 4).

**TABLE 3.**  
**Table 3. Results for Tukey leaching N-NO3 between al treatments (A0-D2).**

Tukey HSD test, NO3, Mean Squarev (Error0) 0.006. The mean difference is significant at the 0.05 level												
	A0	A1	A2	B0	B1	B2	C0	C1	C2	D0	D1	D2
A0		1.000	1.000	0.047	0.035	0.976	0.037	0.003	0.018	1.000	1.000	0.218
A1	1.000		1.000	0.05	0.038	0.980	0.039	0.004	0.020	1.000	1.000	0.231
A2	1.000	1.000		0.052	0.039	0.982	0.041	0.004	0.021	1.000	1.000	0.237
B0	0.074	0.050	0.052		1.000	0.460	1.000	0.990	1.000	0.048	0.055	1.000
B1	0.035	0.038	0.039	1.000		0.383	1.000	0.996	1.000	0.035	0.041	0.998
B2	0.976	0.980	0.982	0.460	0.383		0.395	0.063	0.246	0.977	0.984	0.896
C0	0.037	0.039	0.041	1.000	1.000	0.395		0.996	1.000	0.037	0.043	0.999
C1	0.003	0.004	0.004	0.990	0.996	0.063	0.996		1.000	0.004	0.004	0.744
C2	0.018	0.020	0.021	1.000	1.000	0.246	1.000	1.000		0.019	0.022	0.984
D0	1.000	1.000	1.000	0.048	0.035	0.977	0.037	0.004	0.019		1.000	0.220
D1	1.000	1.000	1.000	0.055	0.041	0.984	0.043	0.004	0.022	1.000		0.246
D2	0.218	0.231	0.237	1.000	0.998	0.896	0.999	0.744	0.984	0.220	0.246	

A0: Control, A1: 1.5 kg m-3 TerraCottem, A2: 3.0 kg m-3 TerraCottem, B0: Glucose (1%), B1: Glucose (1%) and 1.5 kg m-3 TerraCottem, B2: Glucose (1%) - 3.0 kg m-3 TerraCottem, C0: nitrogen fertilizer (50 kg N ha-1), C1: 1.5 kg m-3 TerraCottem and nitrogen fertilizer (50 kg N ha-1), C2: 3.0 kg m-3 TerraCottem and nitrogen fertilizer (50 kg N ha-1), D0: Glucose (1%) + nitrogen fertilizer (50 kg N ha-1), D1: 1.5 kg m-3 TerraCottem and Glucose (1%) + nitrogen fertilizer (50 kg N ha-1), D2: 3.0 kg m-3 TerraCottem and Glucose (1%) + Nitrogen fertilizer (50 kg N ha-1) Red numbers indicate significant difference between the treatments (p<0.05).

**TABLE 4.**  
**Table 4. Statistical analysis of leaching of NH4 between al treatments**

Tukey HSD test, NH4, Mean Squarev (Error0) = 0.227. The mean difference is significant at the 0.05 level												
	A0	A1	A2	B0	B1	B2	C0	C1	C2	D0	D1	D2
A0		1.000	1.000	0.066	0.159	0.976	0.622	0.057	0.013	1.000	1.000	0.820
A1	1.000		1.000	0.062	0.151	0.972	0.604	0.054	0.012	1.000	1.000	0.806
A2	1.000	1.000		0.062	0.150	0.971	0.602	0.054	0.012	1.000	1.000	0.804
B0	0.066	0.062	0.062		1.000	0.559	0.959	1.000	1.000	0.093	0.068	0.847
B1	0.159	0.151	0.150	1.000		0.820	0.998	1.000	0.986	0.215	0.164	0.976
B2	0.976	0.972	0.971	0.559	0.820		0.999	0.519	0.187	0.992	0.978	1.000
C0	0.622	0.604	0.602	0.959	0.998	0.999		0.945	0.624	0.725	0.633	1.000
C1	0.057	0.054	0.054	1.000	1.000	0.519	0.945		1.000	0.082	0.059	0.816
C2	0.013	0.012	0.012	1.000	0.986	0.187	0.624	1.000		0.019	0.013	0.414
D0	1.000	1.000	1.000	0.093	0.215	0.992	0.725	0.082	0.019		1.000	0.893
D1	1.000	1.000	1.000	0.068	0.164	0.978	0.633	0.059	0.013	1.000		0.828
D2	0.820	0.806	0.804	0.847	0.976	1.000	1.000	0.816	0.414	0.893	0.828	

(A0-D2). A0: Control, A1: 1.5 kg m-3 TerraCottem, A2: 3.0 kg m-3 TerraCottem, B0: Glucose (1%), B1: Glucose (1%) and 1.5 kg m-3 TerraCottem, B2: Glucose (1%) - 3.0 kg m-3 TerraCottem, C0: nitrogen fertilizer (50 kg N ha-1), C1: 1.5 kg m-3 TerraCottem and nitrogen fertilizer (50 kg N ha-1), C2: 3.0 kg m-3 TerraCottem and nitrogen fertilizer (50 kg N ha-1), D0: Glucose (1%) + nitrogen fertilizer (50 kg N ha-1), D1: 1.5 kg m-3 TerraCottem and Glucose (1%) + nitrogen fertilizer (50 kg N ha-1), D2: 3.0 kg m-3 TerraCottem and Glucose (1%) + Nitrogen fertilizer (50 kg N ha-1) Red numbers indicate significant difference between the treatments (p<0.05).

Lower leaching was found in the variants without additives (A1, A2). Thus, in agreement with our previous measurements, the most limiting factor for soil microbiota in the Mediterranean area was the soil water content. A similar trend was found in the variant, which contained fertilizers and glucose together and TerraCottem in the recommended doses (D1). In this variant, we applied a source of carbon as well as a source of nitrogen, offering building blocks and a source of energy to soil microbiota. This can result in clean grooving of soil consortium

without losing nitrogen. However, in the variant D2, an increase of nitrogen leaching was observed. In this variant, double doses of TerraCottem were used.

We applied only glucose to the variants B1 and B2. In comparison to the control sample, the leaching increased in these treatments. Soil microorganisms had abundant available carbon substrate but they were limited in nitrogen. This fact could be the cause of the enhancement of nitrogen. The polymer TerraCottem contains nitrogen that can be used by microbiota. Thus, leaching was decreased with the increase of the TerraCottem concentration.

In the last variant C, which contained glucose and TerraCottem (C1, C2), we could see an opposite trend than in the variant B. When we applied higher doses TerraCottem, and therefore additional nitrogen, leaching decreased.

## DISCUSSION

**Effect of carbon and nitrogen addition on soil respiration.** In order to determine the soil respiration rate without the hydropolymers, soil microorganisms were stimulated either by addition of carbon (B0), nitrogen (C0), or both, C and N (D0).

The highest values of respiration in the first week could be partially explained by the “Birch effect” which describes a transient pulse of “in situ” soil CO<sub>2</sub> efflux when dry soils are wetted. Soil in this experiment was taken during a dry Mediterranean season. Unger et al (17) observed the Birch effect on soil microbiota when dry Mediterranean soil was moistened by first rainfall after the dry season. After the first wetting, soil respiration in all treatments became more or less stable (Figure 2). This possibly reflect the decreasing or limiting availability of the key sources (18). The higher soil CO<sub>2</sub> release after glucose addition, which is a readily decomposable material for soil microorganisms, is a well-known phenomenon (9). Nevertheless, glucose addition as a stimulating agent for soil microorganisms did not significantly increase soil respiration in our experiment. Interestingly the addition of nitrogen (C0) decreased soil respiration in the first week compared to the control treatment (A0), but the cumulative respiration during the whole month in this treatment was slightly higher than the that in A0. The slight inhibition of soil respiration in the first week could be a result of insufficient synthesis of various energy-consuming oxidative enzymes after the addition of N leading to the limitation of substrate resources and a decrease in soil respiration (19). If the reduced activities of soil microorganisms induced by the solely N addition were eliminated by the simultaneous addition of glucose (D0), then the cumulative respiration would be significantly higher compared to all other treatments. These results support the idea that C and N availability as well as the moisture oscillations are tightly coupled with key factors that are influencing heterotrophic respiration of soil microorganisms (20).

However, in general terms, our results suggest that additional carbon and nitrogen applied to this Mediterranean soil do not significantly promote soil microbial activity. Similar results have been reported in a previous study for dry lands by Zohuriaan-Mehr et al (11).

**Leaching of mineral nitrogen.** According to Binkley et al (15), the leaching of mineral nitrogen is a useful indicator of soil quality. In a natural ecosystem, the leaching of mineral nitrogen would take place only occasionally and in limited amounts. Only in such circumstances, when internal capacities of soils to immobilize nitrogen are overloaded, the leaching of ammonium and nitrate nitrogen can appear. The output of mineral nitrogen with soil percolates is a natural mechanism to balance the offer of nitrogen

in the whole soil profile e.g. when protein accumulation after death of an animal occurs (21). In case of overdoses of nitrogen fertilizers in arable soils, the leaching of mineral nitrogen, as a balancing mechanism of nitrogen cycle, could appear, which can be dangerous for parameters of seepage water and for the quality of the groundwater, especially in the water protection zones (15). In our study, lower rates of mineral nitrogen leaching occurred in the control sample (A0) (Figure 3). However, similar rates of leaching have been previously reported for Mediterranean soils (22). Lower leaching in this variant could be explained by the C/N ratio. In our soil, the C/N ratio was high (Table 1) and net nitrogen mineralization of organic material was very low, because soil microbiota is limited by an insufficient amount of nitrogen concentration. Anabolic microbial reactions, which produce new organic nitrogen compounds, effectively preserve the movement as well as leaching of mineral nitrogen in soils. These assumptions have been also reported by Cleveland et al (23).

Similar effects of induced imbalances between the availability of carbon sources for microbial activities and nitrogen immobilization after addition of nitrogen have been also described in other studies (24).

**The effect of TerraCottem on soil respiration.** Based on our results, addition of carbon, nitrogen and both biogenic elements simultaneously did not affect the target soil as strong as the soil moisture. This indicates that soil water is likely the main controlling factor for Mediterranean soil microbiota (Figure 2). However, in Mediterranean areas the lack of rainfall can represent a critical problem for remaining soil activities (25).

Previous studies have shown that amending soil with polymers, including gypsum and polyacrylamide (PAM), increases the soil water content and reduce runoff and erosion (26). Specifically, Hueso-González et al (1) reported that addition of TerraCottem to soil in Mediterranean areas results in wetter soil, which enhances the process of water infiltration and increases soil moisture contents in the 0-10 cm soil depth layer. However, how the application of this polymer can affect soil microbial activities remained so far unknown for Mediterranean soils.

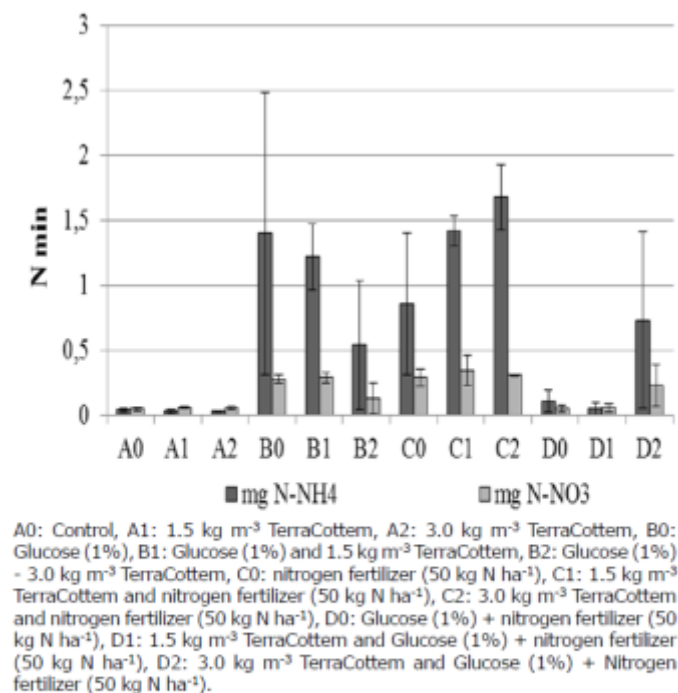
In this study, we tested two different doses of TerraCottem (Table 2). Results for the recommended dose (A1, B1, D1) did not show a significant increase of the cumulative production of CO<sub>2</sub> in comparison to the treatments without TerraCottem addition (A0, B0, D0) (Figure 4). Only in the treatment with combined additions of TerraCottem and a nitrogen fertilizer we observed a decreased in the the cumulative soil respiration. This implies that avoiding a direct application of mineral nitrogen, an amount of 1.5 kg/m<sup>3</sup> of TerraCottem could increase soil moisture in Mediterranean soils, as others authors previously reported Hueso-González et al (1), and it could be harmless for soil microbiota. However, the application of the double amount of TerraCottem caused a decrease in the soil respiration values compared to the A0 control treatment (Figure 4).

TerraCottem is a mix of copolymers with acrylamide and acrylic salt with potash (39.5%), fertilizers (with 10.5% amount of nitrogen) and a carrier material to further enhance the root and plant growth and to reduce inputs of key nutrients (9). These polymers increased their volume during moistening, and formed the continuum from the solid to gel-like state that had a binding-sealing effect in the soil. Similar results have been found in situ after rainfall events in Mediterranean forestall soils (1). The decrease of soil respiration, which was recorded in the A2 treatment, would probably disappear after the adaptations of soil microbiota (27).

Similar patterns were found in soils amended with glucose (B0, B1 and B2) and with a mixture of glucose and a nitrogen fertilizer (D0, D1 and D2) (Figure 4). Opposite, a different pattern was found when soil was treated with a nitrogen fertilizer, which can be explained by the reaction of soil microbiota on the supply of an easily available source of nitrogen (25).

**The effect of TerraCottem on nitrogen leaching.** According to our results (Figure 5), the application of TerraCottem into the soil has not affected the leaching of ammonium or nitrate nitrogen. Thus, from

the conservation management standpoint, polymers could be a useful tool for reduction of the plant stress during a Mediterranean dry season without an impact on losing nitrogen from the soil.



**Figure 5.** The leaching of nitrogen from different treatments of experimental soil.

FIGURE 5  
Figure 5

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