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Carbon and nitrogen mineralisation from green manures as alternative nitrogen sources in Mediterranean farming

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ABSTRACT

The soil incorporation of green manures is a practice that can be used in sustainable agriculture and in organic farming, where nitrogen (N) sources are limited. The aim of this study was to evaluate balansa clover (*Trifolium michelianum* Savi), yellow lupine (*Lupinus luteus* L.) and ryegrass (*Lolium multiflorum* Lam.) as potential alternative N sources. A total of nine treatments were considered in this study: control, aerial of balansa clover, roots of balansa clover, aerial of yellow lupine, roots of yellow lupine, aerial of ryegrass, roots of ryegrass, mixture aerial + roots of yellow lupine and mixture aerial + roots of ryegrass. A laboratory incubation experiment was conducted under controlled conditions during 196 days and carbon and N mineralisation were followed. Results showed that green manures are appropriate N sources for Mediterranean farming. No significant differences in terms of N mineralisation were observed between aerial or roots biomass of the green manures. Besides, 37–55% of total N applied was mineralised in treatments amended with balansa clover or yellow lupine, whereas 13–21% of total N applied was mineralised in ryegrass. It can be concluded that the most efficient green manure for supplying mineral N to the succeeding crop was yellow lupine.

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Introduction

In Mediterranean farming, leguminous crops can fix atmospheric nitrogen (N) symbiotically and supply N to the subsequent crops, when these crops are included in rotations during autumn–winter months (Jacobsen et al. 2012; Dalias 2015). Moreover, legumes contribute to improve soil organic matter content, soil structure and availability of soil phosphorus (Preissel et al. 2015).

The incorporation of legumes as green manures into the soil is a technique that can be used in sustainable agriculture and in organic farming, where N sources are scarce (Arrobas et al. 2016; Perdigão et al. 2012). In addition, when legumes are used as a break crop in rotations dominated by grasses (cereals) or plants from other botanical families, they play an important role in the pest and diseases suppression by interruption of life cycles of plant enemies (Fan et al. 2006).

Previous studies (Arrobas et al. 2016; Frankenberger Abdelmagid 1985; Nakhone & Tabatabai 2008; Justes et al. 2009; Perdigão et al. 2012; Carranca et al. 2015) reported a high potential of legumes for supplying mineral N throughout mineralisation to the subsequent crop. However,

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legumes being a potential N source (as green manures) for the succeeding main crop will need a previous evaluation of their effects on soil carbon (C) and N decomposition kinetics (Cayuela et al. 2009). C and N mineralisation of green manures are controlled by several factors such as C/N ratio and labile C fractions (Gutser et al. 2005; Ribeiro et al. 2010) and varied among soils for a particular green manure. In addition, Carranca et al. (2015) reported that the above-ground N concentration did not vary among legumes, but differed in the below-ground tissues and consequently the role of legume roots for soil N fertility is underestimated.

With the development of a new generation of forage legumes (Loi et al. 2005; Nichols et al. 2007), some species such as balansa clover and yellow lupine have not yet been tested as a potential N source on Mediterranean environment. More experimental data are required in order to evaluate the influence of biochemical characteristics of green manures on C and N mineralisation, namely the potential benefits of residues management on soil quality and fertility (Moreno-Cornejo et al. 2014). The aim of this study was to evaluate balansa clover (*Trifolium michelianum* Savi), yellow lupine (*Lupinus luteus* L.) and ryegrass (*Lolium multiflorum* Lam.) as green manures to be used as potential alternative N sources in Mediterranean farming.

Material and methods

Green manures and soil used

The green manures used in this study were collected in full bloom from a field experiment (latitude: 40.641786º, longitude: −7.911335º) previously made between September (sowing) and April 2012 (harvest) at Agrarian School of Viseu (Viseu, Portugal). Considering the results reported by Perdigão et al. (2012) in a two-year field study, nine winter annual green manures were tested, and we selected the following three green manures to be evaluated in this study, namely balansa clover (*Trifolium michelianum* Savi) Cv. Paradana (seeding rate of 10 kg ha^{-1}), yellow lupine (*Lupinus luteus* L.) regional population (seeding rate of 60 seeds m_{-2}) and ryegrass (*Lolium multiflorum* Lam.) Cv. Liforia (seeding rate of 30 kg ha^{-1}). Mineral or organic fertilisers were not applied during the growing of the green manures. Additional information relative to the agronomic practices could be found in Perdigão et al. (2012) and the weather conditions during the growing period of the green manures are shown in Figure 1. After harvest (April 2012), the green manures were oven-dried at room temperature, separated in aerial biomass and roots, grossly grinded and lyophilised. Then, green manures were finely grinded and a subsample was analysed for physico-chemical characterisation (Table 1).

The soil used in this study was classified as a Dystric Fluvisol (WRB 2015), with a sandy-loam texture (44.2% coarse sand, 24.0% fine sand, 16.3% silt and 15.4% clay), being collected in July 2012

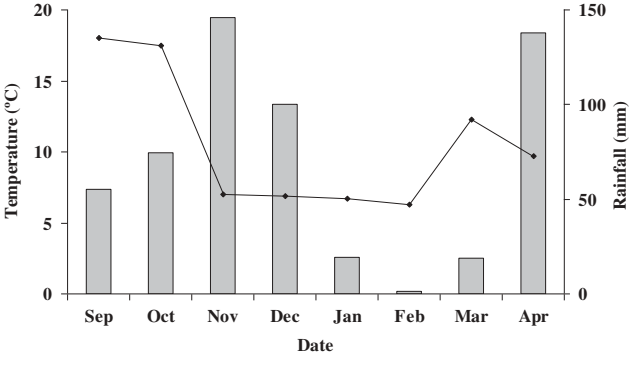

Figure 1. Average air temperature (line) and rainfall (columns) during the cultivation of the green manures.

Table 1. Physico-chemical characterization of the green manures studied.

Green manures	Acronym	Dry matter (g kg^{-1})	Total C (g kg^{-1} DM)	Total N (g kg^{-1} DM)	C / N	Application rate mg C kg^{-1} dry soil	Application rate mg N kg^{-1} dry soil
Aerial balansa clover	ABC	5.7	396	24.5	16	2259	140
Root balansa clover	RBC	6.0	328	16.6	20	1975	100
Aerial yellow lupine	AYL	3.5	446	40.3	11	1544	140
Root yellow lupine	RYL	4.8	424	29.1	15	2045	140
Aerial ryegrass	AR	7.7	386	18.1	21	2986	140
Root ryegrass	RR	15.6	203	8.9	23	3178	140
Mixture aerial + root yellow lupine	MYL	3.7	441	37.4	12	1648	140
Mixture aerial + root ryegrass	MR	10.5	299	13.3	22	3055	140

DM: dry matter.

from the upper layer (0–200 mm) in the nearby area where green manures were produced. The physico-chemical properties of the soil include the following: bulk density: 0.9 g cm^{-3}, pH (H_2O): 6.0, electrical conductivity: 0.02 mS cm^{-1}, water holding capacity (WHC) at pF 2.0, 38.4% (w/w), total C: 15.60 g kg^{-1} dry soil, total N: 1.84 g kg^{-1} dry soil, NH_4^+–N: 0.0 mg kg^{-1} dry soil, NO_3–N: 66.0 mg kg^{-1} dry soil. At laboratory, the soil was manually sieved (<2 mm), homogenised and moisture was corrected with deionised water to 60% WHC. Then, soil was stored at 4°C for 1 month before beginning the study.

A detailed description of the methods used to assess the physical–chemical properties of the soil and green manures samples can be found in Ribeiro et al. (2010). Briefly, total C and total N were determined using an elemental analyser by Dumas (Primacs^{SC}, Skalar, Breda, Netherlands) and near infrared detection (SanPlus, Skalar).

Experimental design

In September 2012, three soil incubations were performed simultaneously under controlled conditions (25°C and 60% WHC) to follow the (i) soil N dynamics, (ii) carbon dioxide (CO_2) emissions and (iii) ammonia (NH_3) emissions in soil amended with the eight treatments.

A total of nine treatments with four replicates were considered in this study: (i) non-amended soil (Control); (ii) aerial biomass of balansa clover (ABC); (iii) roots biomass of balansa clover (RBC); (iv) aerial biomass of yellow lupine (AYL); (v) roots biomass of yellow lupine (RYL); (vi) aerial biomass of ryegrass (AR); (vii) roots biomass of ryegrass (RR); (viii) mixture aerial + roots biomass of yellow lupine (MYL); (ix) mixture aerial + roots biomass of ryegrass (MR).

An amount of 50 g dry soil (depth = 50 mm) was packed into PVC containers (Ø = 25 mm, H = 70 mm). Green manures were manually applied and homogeneously mixed in soil at a rate of 140 mg N kg^{-1} dry soil, except in RBC treatment where a rate of 100 mg N kg^{-1} dry soil was added (Table 1). The addition of the green manure residues (aerial, roots or mixture of both) has the same proportion that plants have at field conditions. The PVC containers were maintained at 25°C and 60% WHC during the whole experiment. Soil moisture was checked periodically (twice a week) by weighing the PVC containers and when necessary was corrected to 60% WHC by deionised water addition.

Carbon and ammonia fluxes

Carbon dioxide fluxes were measured following the procedure described by Kabwe et al. (2002) and used by Fangueiro et al. (2010). The measurement of CO_2 fluxes was carried out in each treatment at days 1, 2, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, 56, 70, 84, 98, 112, 126, 140, 154 and 168. A PVC container of each treatment was placed in a Kilner jar (L = 100 mm, H = 210 mm) together with

a beaker containing 20 mL 1 M NaOH to trap CO_2. This solution in each trap was replaced in each sampling date. After 30 min of aeration, a new trap was placed in each Kilner jar, and the jar was immediately closed. The solutions collected were analysed for CO_2 evolved by a titrimetric determination method (Zibilske 1994).

In each treatment and along the incubation period, the cumulative C mineralised ($C_{Released}$) was calculated by Equation (1):

$$C_{Released} = \left(\frac{C_{Treatment(t)} - C_{Control(t)}}{C_{Applied}}\right) \times 100, \quad (1)$$

where $C_{Released}$ is the percentage of CO_2–C emitted at time t (mg C g^{-1} applied C), $C_{Treatment(t)}$ is the percentage of C emitted by each treatment at time t (mg C), $C_{Control(t)}$ is the percentage of C emitted by Control at time t (mg C) and $C_{Applied}$ is the amount of organic C applied in each treatment (g C).

The CO_2–C fluxes (C_{Flux}) were determined using Equation (2):

$$c_{Flux} = \frac{c_{(t2)} - c_{(t1)}}{t2 - t1}, \quad (2)$$

where C_{Flux} is the CO_2–C emitted by each treatment at incubation interval *t1–t2* (mg CO_2–C kg^{-1} dry soil day^{-1}), $C_{(t2)}$ and $C_{(t1)}$ are the CO_2–C emitted by each treatment at the end and at the beginning of the incubation interval *t*1–*t*2, respectively (mg CO_2–C kg^{-1} dry soil), and *t*1–*t*2 is the incubation interval used to determinate CO_2–C fluxes (days).

Ammonia fluxes were measured during the first 14 days of experiment using the acid traps method as used by Fangueiro et al. (2015). An acid trap, containing 10 mL of 0.05 M orthophosphoric acid, was placed together with a PVC container of each treatment inside the Kilner jars. Acid solution in each trap was replaced after 1, 3, 7 and 14 days of the beginning of the experiment. Acid solutions collected at each sampling date were analysed for NH_4^+–N content using automated segmented-flow spectrophotometry (Houba et al. 1995). Cumulative losses were estimated by averaging the flux between two sampling occasions and multiplying by the time interval between the measurements.

Nitrogen mineralisation

On days 0, 1, 3, 7, 10, 14, 21, 35, 56, 84, 112, 154 and 196 after the beginning of the experiment, four PVC containers of each treatment were used to assess soil mineral N content (NH_4^+–N and NO_3^-–N). Soil mineral N content was assessed by extraction with 2 M KCl at a 1:5 ratio and followed by molecular-absorption spectrophotometry using the Berthelot and sulfanilamide methods for NH_4^+ and NO_3^-, respectively (Houba et al. 1995). The net N mineralisation (NNM) rates were calculated according to Equation (3):

$$NNM_{(t)} = \text{Mineral } N_{(t)} - \text{Minera l}N_{(t=0)}, \quad (3)$$

where $NNM(_t)$ is net N mineralisation at incubation interval t (days) in the different treatments (mg N kg^{-1} dry soil day^{-1}), Mineral $N(_t)$ and Mineral $N(_{t=0})$ are the amount of soil mineral N in the different treatments at the end and at the beginning of the incubation interval, respectively (mg N kg^{-1} dry soil).

The total N mineralisation (TNM) was calculated according to Equation (4):

$$TNM = \left(\frac{NNM_{Treatment} - NNM_{Control}}{N_{Applied}}\right) \times 1000, \quad (4)$$

where TNM is total N mineralisation over the incubation (mg N g^{-1} applied N) in amended treatments, $NNM_{Treatment}$ is the total N mineralised at the end of experiment in amended

treatments (mg N), $NNM_{Control}$ is the total N mineralised at the end of experiment in Control and $N_{Applied}$ is total N applied in each treatment (g N).

Statistical analysis

Results obtained were analysed by one-way analysis of variance (software SPSS statistics 17.0, Chicago, IL, USA) to test the effects of each treatment and time independently. The comparison of means between treatments was assessed by Duncan *t*-test at $p < 0.05$.

Carbon mineralisation data were fitted exponentially, using the following one-pool first-order kinetic equation (Stanford & Smith 1972), as described in Equation (5):

$$C_{min} = C_0 \times \left(1 - \exp^{(-k \times t)}\right), \quad (5)$$

where C_{min} is cumulative C mineralised (mg C g^{-1} applied C) at time t (days), C_0 is the potentially mineralisable organic C (mg C g^{-1} applied C), k is mineralisation rate constant (day^{-1}) and t is the time from the start of the incubation (days).

Results

Carbon mineralisation

As can be observed in Table 2, the CO_2 fluxes peaked in the first 7 days of incubation in all amended treatments. Besides, 45% of the cumulative CO_2 losses in treatments with legumes (ABC, RBC, AYL, RYL and MYL) occurred in first 5 days of incubation. However, no significant differences were found ($p > 0.05$) in cumulative CO_2 losses between treatments amended with aerial and roots biomass of a particular green manure (Table 2).

The cumulative amounts of applied C mineralised from the green manures are shown in Figure 2. The amount of C mineralised in MR treatment was about five times higher when compared with Control (Table 2). In the first 3 days of incubation, significant lower ($p < 0.05$) amounts of applied C mineralised in AYL, RYL and MYL treatments were observed relative to all other treatments. At the end of the incubation, the amounts of applied C mineralised were significantly higher ($p < 0.05$) in RBC and MR treatments comparatively to all other treatments. The cumulative amounts of applied C mineralised ranged from 52% in RYL treatment to 70% in MR treatment (Figure 2).

Results from the fitting the exponential model applied to the percentages of applied organic C in green manures are shown on Table 3. As can be seen, the C_0 corresponds to 65% of the C applied in MR treatment against <56% in all other treatments. The lower values of k and the higher value of $t_{1/2}$ were observed in RR and MR treatments (Table 3).

Table 2. Fluxes (mg CO_2–C kg^{-1} dry soil day^{-1}) and cumulative CO_2 emitted (mg CO_2–C kg^{-1} dry soil) during the experiment from green manures ($n = 4$).

	Treatments								
Time interval (days)	Control	ABC	RBC	AYL	RYL	AR	RR	MYL	MR
0–1	5^{e}	212^{a}	188^{ab}	147^{cd}	187^{ab}	173^{bc}	171^{bc}	135^{d}	166^{bc}
2–7	5^{g}	104^{bc}	86^{de}	65^{f}	78^{ef}	114^{b}	96^{cd}	70^{f}	136^{a}
10–14	5^{e}	17^{c}	18^{c}	12^{d}	19^{c}	34^{b}	35^{b}	17^{c}	42^{a}
21–27	6^{g}	9^{de}	10^{d}	6^{fg}	9^{def}	17^{c}	21^{b}	7^{efg}	25^{a}
36–84	4^{cd}	5^{bc}	4^{cd}	2^{e}	3^{cde}	5^{bc}	6^{b}	2^{de}	8^{a}
98–168	3^{a}	2^{bc}	$2b^{c}$	1^{a}	1^{bc}	1^{bc}	2^{ab}	1^{a}	2^{ab}
Σ 0–168	557^{f}	1886^{c}	1797^{cd}	1363^{e}	1689^{d}	2173^{b}	2299^{b}	1475^{e}	2698^{a}

Values with different superscript letters within rows are significantly different ($p < 0.05$) by Duncan test.

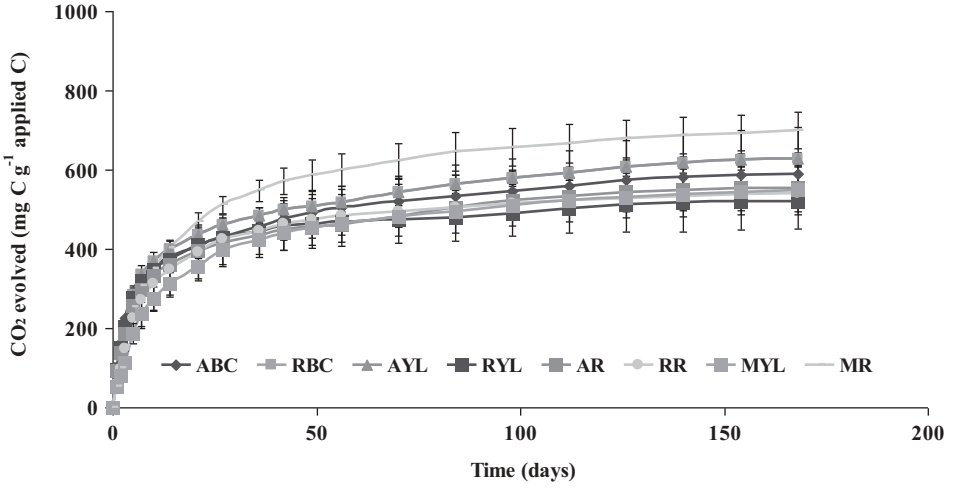

Figure 2. Cumulative amounts of applied C mineralised evolved as CO_2–C from the green manures (vertical bars represent standard deviation of four replicates).

Table 3. Model parameters estimated using the first-order exponential model applied to the percentages of applied organic C released as CO_2–C from green manures.

	Model parameters			
Green manures	C_0	k	r^2	Half-life time (days)
Aerial balansa clover (ABC)	0.52	0.126	0.93	5
Root balansa clover (RBC)	0.56	0.111	0.98	6
Aerial yellow lupine (AYL)	0.48	0.150	0.96	5
Root yellow lupine (RYL)	0.50	0.115	0.94	6
Aerial ryegrass (AR)	0.50	0.093	0.98	7
Root ryegrass (RR)	0.51	0.068	0.99	10
Mixture yellow lupine (MYL)	0.50	0.124	0.97	6
Mixture ryegrass (MR)	0.65	0.070	0.98	10

C_0 is potentially mineralisable organic C (mg C kg^{-1} applied C), k is mineralisation rate constant (day^{-1}) and r^2 is the coefficient of determination.

Nitrogen mineralisation

As can be observed in Table 4, very small NH_3 losses (≤8 μg NH_3^-–N kg^{-1} dry soil) were observed during the whole measurement period in all treatments.

The evolution of NH_4^+ and NO_3^- concentration in each treatment is shown in Figure 3. The initial concentrations of NH_4^+ were significantly increased ($p < 0.05$) in amended treatments relative to Control (Figure 3(a,b)). The amendment of RYL and AYL treatments led to an increase of the NH_4^+ content close to 24 mg NH_4^+–N kg^{-1} dry soil in RYL and AYL treatments, against 15 mg NH_4^+–N kg^{-1} dry soil in MR treatment (Figure 3(a,b)). The NH_4^+ concentration reached the Control values after 7 days of incubation in all amended treatments (Figure 3(a,b)).

Table 4. Fluxes (μg NH_3–N kg^{-1} dry soil day^{-1}) and cumulative NH_3 emitted (μg NH_3–N kg^{-1} dry soil) during the experiment from green manures ($n = 4$).

	Treatments								
Time interval (days)	Control	ABC	RBC	AYL	RYL	AR	RR	MYL	MR
0–7	0^d	0^d	0^d	0^d	8^a	0^d	1^c	2^b	0^d
8–14	0^b	3^a	0^b	0^b	0^b	0^b	0^b	0^b	0^b
Σ 0–14	0^e	3^b	0^e	0^e	8^a	0^e	1^d	2^c	0^e

Values with different superscript letters within rows are significantly different ($p < 0.05$) by Duncan test.

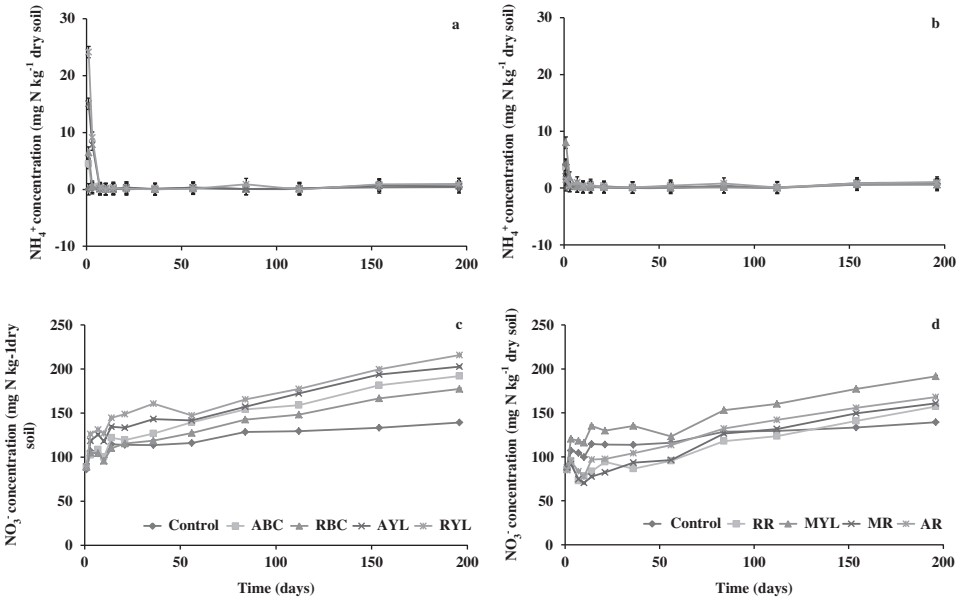

Figure 3. Evolution of the soil $NH4^+$ (A and B) and $NO3^-$ (C and D) concentrations in the treatments (vertical bars represent standard deviation of four replicates).

The initial NO_3^- concentration was about 88 mg NO_3^-–N kg^{-1} dry soil in all treatments including the Control (Figure 3(c,d)). The NO_3^- concentration increased during the incubation in all treatments (157 and 216 mg NO_3^-–N kg^{-1} dry soil for RYL and RR treatments, respectively), being significantly higher ($p < 0.05$) in RYL, AYL and MYL treatments (Figure 3(c,d)).

Figure 4 shows NNM of the green manures during the incubation. As can be seen, N immobilisation occurred in the first 10 days in all amended treatments, except in AYL, RYL and MYL treatments in which N mineralisation was significantly higher ($p < 0.05$) during all incubation (Figure 4(a,b)). Nitrogen mineralisation was observed after 14 days of incubation in ABC and RCB treatments, whereas in AR, RR and MR treatments mineralisation was observed only after 84 days of incubation (Figure 4(a,b)).

Over the whole incubation, no significant differences ($p > 0.05$) in terms of N mineralisation were observed between treatments receiving aerial or roots of a particular green manure (Figure 5). Also, no significant differences were observed ($p > 0.05$) between treatments with legumes (balansa clover and yellow lupine), although numerically higher differences were observed for

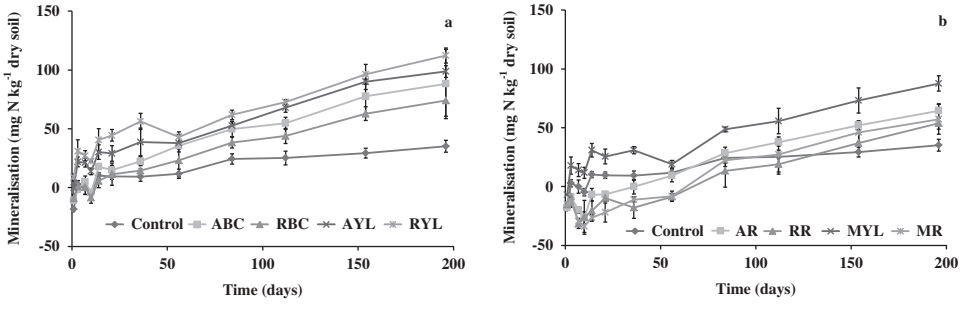

Figure 4. Net N mineralisation of the green manures over the incubation (vertical bars represent standard deviation of four replicates) .

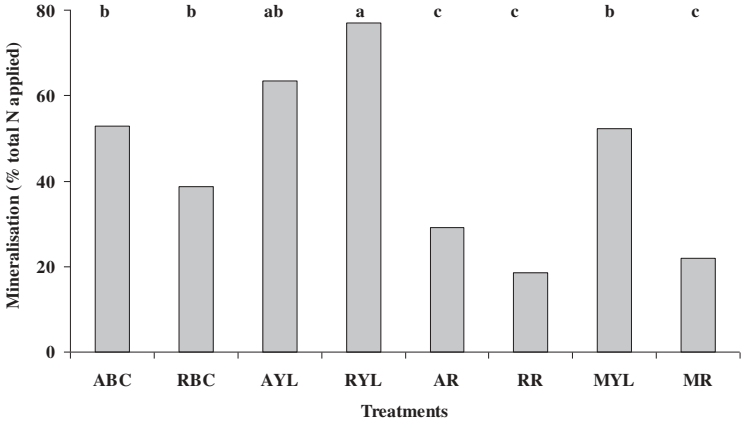

Figure 5. Apparent N mineralisation of the green manures in function of total N applied at the end of the incubation. Values presented with different letters are significantly different ($p < 0.05$) by Duncan test.

yellow lupine (ca. 12% higher) (Figure 5). Apparent N mineralisation was significantly higher ($p < 0.05$) in treatments with legumes (ABC, RBC, AYL, RYL and MYL) relative to treatments with non-legumes (AR, RR and MR) (Figure 5). In addition, 37–55% of total N applied was mineralised in treatments amended with legumes, whereas 13–21% of total N applied was mineralised in treatments with non-legumes (Figure 5).

Discussion

Predicting C and N mineralisation of plant residues returned to soil is very important for soil N availability (Hassan 2013). The higher CO_2 fluxes observed in the 7 days of experiment (Table 2) could be related with the presence of easily decomposable substrates in green manures (Moreno-Cornejo et al. 2014). Thus, the application of the three green manures (balansa clover, yellow lupine and ryegrass) to soil increased C mineralisation about five times, in agreement with previous studies (Gutser et al. 2005; Raiesi 2006; Justes et al. 2009; Zeng et al. 2010; Hassan 2013) who reported that the application of organic matter enhances soil respiration.

As can be seen in Table 3, the lower values of C_0 and k and the higher value of $t_{1/2}$ in treatments with ryegrass are related with higher recalcitrant C fractions contained in this green manure relative to balansa clover and yellow lupine, which should have high contents of easily degradable C compounds (e.g. labile C fractions). Redin et al. (2014) analysed 25 aerial biomass residues and the C_0 values of aerial biomass varied from 0.47 to 0.64, being in line with our study. The cumulative amounts of applied C mineralised ranged from 52% to 70% in the three green manures (Figure 2). Our results are in agreement with Justes et al. (2009) who reported cumulative amounts of applied C mineralised between 59% and 68% in green manures.

Previous studies (Nakhone & Tabatabai 2008; Li et al. 2013) reported that legumes mineralised more rapidly than non-legumes. The reasons that balansa clover and yellow lupine mineralised more rapidly than ryegrass are related with higher total N content and lower C/N ratio in balansa clover and yellow lupine (Table 1). In addition, aerial biomass from balansa clover and yellow lupine had similar N mineralisation rates, but roots from balansa clover had a mineralisation rate 50% lower than roots from yellow lupine (Figure 5). The lower mineralisation of roots from balansa clover than from yellow lupine could be related with the following: (i) lower total N content and high C/N ratio (Table 1); (ii) presence of lignoprotein complexes that are resistant to microbial decomposition and (iii) toxic metabolites that could inhibit mineralisation (Frankenberger & Abdelmagid 1985). Other factors such as lignin, polyphenols, proteins, soluble carbohydrates,

cellulose and hemicelluloses contents could also explain differences on C and N mineralisation among the three green manures (Nakhone & Tabatabai 2008).

The efficiency of the studied green manures for supplying mineral N for the subsequent crop increases by the following order: yellow lupine > balansa clover > ryegrass. Nevertheless, our results were obtained under laboratory conditions and need to be validated under field conditions.

Conclusion

Our laboratory study showed that green manures (balansa clover, yellow lupine and ryegrass) are good N sources for Mediterranean farming. No significant differences in terms of N mineralisation were observed between aerial or roots biomass of the green manures. Besides, 37–55% of total N applied was mineralised in treatments amended with balansa clover or yellow lupine, whereas 13–21% of total N applied was mineralised in ryegrass. It can be concluded that the most efficient green manure for supplying mineral N to the succeeding crop was yellow lupine. Further field studies will be required to validate our results.

Disclosure statement

No potential conflict of interest was reported by the authors.

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