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RESEARCH ARTICLE

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Nitrogen mineralization of legume residues: interactions between species, temperature and placement in soil

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Abstract

Aim of study: To assess the interactive effects of legume species, residue placement and temperature on the net nitrogen (N) mineralization dynamics in a sandy loam soil.

Area of study: Northern Portugal.

Material and methods: Cowpea (*Vigna unguiculata* L. Walp), faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) residues were incorporated or applied to the soil surface at typical field yields in Europe and incubated in aerobic conditions for up to 240 days, either at 10 °C or 20 °C. Initial chemical characteristics of the soil and residues were determined. Net N mineralization was estimated at eight time intervals.

Main results: Cowpea residues caused no negative changes in soil mineral N contents and were able to release the equivalent of 21-45 kg N ha^{-1} in 240 days. Net N immobilization (up to 17 kg N ha^{-1}) was observed throughout most of the trial in soil with faba bean and pea residues. Differences in mineralization patterns could be attributed to the higher quality (lower carbon to nitrogen (C:N) ratios) of cowpea. Surface placement increased net N mineralized by as much as 18 kg N ha^{-1}. The sensitivity of N mineralization to changes in temperature and residue placement varied with legume species, likely due to effects associated with differences in C:N ratios.

Research highlights: Adding cowpea residues to soil is suitable when high N availability is immediately required. Faba bean or pea residues are better suited for conservation of soil N for later release.

Additional key words: cowpea; faba bean; pea; residue management; microcosm; incubation.

Authors' contributions: Conceived and designed the experiments: MO and HT. Performed the experiments: DR and MO. Acquired the data: MO, DR, JC and LF. Analyzed the data and wrote the manuscript: MO. Critically revised the manuscript for important intellectual content: DR, JC, LF and HT. Obtained funding and supervised the work: HT.

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Introduction

Returning above-ground crop residues to the soil provides many benefits to agroecosystems, such as improved soil physical properties, conserved soil organic matter contents and increased nutrient availabilities (Chen *et al.*, 2014). Pulse crop residues are high in N, which can be supplied to subsequent crops when residues are returned to the soil system. However, the range of this "nitrogen effect" varies with legume species, agricultural management and site (Peoples *et al.*, 2009).

Indeed, nitrogen (N) release from plant residues decomposing in soil results from complex microbial processes controlled by many factors, such as the quality or chemical composition of the residues, which varies with legume genotypes (Trinsoutrot *et al.*, 2000; Peoples *et al.*, 2009). The carbon to nitrogen (C:N) ratio of the plant material has often been used as a predictor of residue mineralization. C:N ratios below 20 generally imply net N mineralization, while C:N ratios above 30 usually result in net N immobilization (Trinsoutrot *et al.*, 2000; Chen *et al.*, 2014). Although this ratio is useful in predicting residue mineralization, estimates based on it are not always accurate, as different compounds containing carbon (C) and N have different stabilities (Chen *et al.*, 2014). Soluble components such as carbohydrates or amino acids are rapidly decomposed, while cellulose and hemicellulose components are more slowly decomposed (Hadas *et al.*, 2004). Lignin-like compounds can slow down the N mineralization rate due to their recalcitrance (Hadas *et al.*, 2004; Frei, 2013) and protective effect on cellulose and hemicellulose decomposition (Pauly & Keegstra, 2008; Bhatnagar *et al.*, 2018).

The location of the residues in soil is also known to affect their N mineralization pattern. When decomposing on the surface, residues have less contact with soil microorganisms (Coppens *et al.*, 2006) and are more liable to moisture limitation (Coppens *et al.*, 2007) than when decomposing below the surface. Accordingly, residue decay rates are usually lower at the soil surface (Coppens *et al.*, 2006; Mulvaney *et al.*, 2017), except when anaerobic conditions occur beneath the surface (Li *et al.*, 2013). Microbial activity is limited by low N availability at the soil surface, particularly in residues with low C:N ratio (Coppens *et al*., 2007). Hence, lower mineral N immobilization occurs when residues are placed on the surface rather than incorporated into the soil (Chen *et al.*, 2014). Indeed, higher soil N availability has generally been reported when residues are applied to the surface of diverse types of soil (Coppens *et al.*, 2006; Abiven & Recous, 2007; Li *et al.*, 2013). The location of residues in soil is linked with tillage system, as incorporation implies tillage operations, while in no-tillage systems residue decomposition occurs at the soil surface. Therefore, differences in net N mineralized under the two systems derive not only from different residue locations but also from differences in soil organic matter mineralization in tilled vs. no-tilled soil due to disruption of aggregates, increased aeration and decreased moisture content (Balesdent *et al.*, 2000).

N mineralization is also affected by environmental factors that regulate the growth and activity of the microbial community, such as temperature (Sierra, 2002; Roberts *et al.*, 2015). A positive effect of temperature on N mineralization is generally recognized, but the optimum temperature for decomposition and its consequences on mineralization kinetics seem to differ with soil characteristics (Roberts *et al.*, 2015). N mineralization has been reported to increase with increasing temperature fluctuations (Sierra, 2002). Decomposer microflora is differently affected by temperature changes in the range 0–20 °C. Fungi have lower optimum temperatures and thus are better adapted to cold conditions than bacteria, which are more susceptible to temperature changes (Siles *et al.*, 2019). Soil pH can also affect mineralization of crop residues (Xiao *et al.*, 2013; Wang *et al.*, 2017). Nitrification rates decrease linearly with decreasing soil pH in the range 4–6.5 (Wang *et al.*, 2017) and practically stop below this range (Xiao *et al.*, 2013). However, ammonification is usually little inhibited by increasing soil acidity (Xiao *et al.*, 2013; Wang *et al.*, 2017).

To our knowledge, few studies have compared interactions between legume species, residue placement and temperature on the net N mineralized in soil. Within this framework, we aimed to test the following hypotheses: i) the above-ground residues from different mature legume crops have different chemical characteristics and thus have distinct net N mineralization patterns when added to soil; ii) these net N mineralization patterns will differ when residues are incorporated or applied to soil surface; and iii) the response of net N mineralization to different temperatures will vary with residue characteristics and placement in soil. With this in mind, we conducted a 240-day laboratory microcosm study with the objectives of measuring and comparing the N mineralized from mature residues of cowpea, faba bean or pea incorporated into the soil or applied to its surface, and incubated at two distinct constant temperatures (10 or 20 °C).

Material and methods

Soil and legume residues

Soil was collected from the surface layer (0-20 cm) of an experimental field in the University of Trás-os-Montes and Alto Douro (41°17’05.5”N 7°44’20.2”W; Vila Real, Portugal). The field had been under conventional tillage and continuously cultivated for rainfed fodder (winter triticale intercropped with oats) in the previous 40 years, and was exceptionally under fallow when soil was collected. The soil was an acidic sandy-loam (68% sand, 22% silt, 10% clay), Dystric Cambisol (IUSS Working Group WRB, 2015), with pH (in water) of 4.9, 2.8% organic matter content, Egner-Riehm's Extractable phosphorus (P) and potassium (K) concentration of 32 and 171 mg kg^{-1}, respectively, 4 $cmol_c$ kg^{-1} effective cation exchange capacity and 37 mg inorganic N kg^{-1} prior to the incubation. Air dried soil was sieved through a 2 mm mesh, mechanically homogenized, moistened to 50% water-filled pore space (WFPS) and pre-incubated at 20 °C for 15 days.

Faba bean (*Vicia faba* L. cv. Favel), pea (*Pisum sativum* L. cv. Grisel) and cowpea (*Vigna unguiculata* (L.) Walp cv. Fradel) crops were grown in a Mediterranean climate in plots adjacent to the agricultural field where soil was collected for the incubation experiment (Oliveira *et al*., 2019). Agricultural lime and fertilizers were applied to improve soil pH and nutrient availability, except N, according to regional recommendations for these crops (a detailed description is available at Oliveira *et al*., 2019). Faba bean and pea crops were rainfed cultivated from Autumn to Spring, while cowpea was Summer-grown with irrigation. Legume grains were harvested at full maturity (pulse). The remaining above-ground biomass (senescent stems and leaves, henceforth named residues) were collected, dried at 50 °C until constant weight (approximately 72 h) and ground to 1 mm particle size in a rotor mill (Retsch Cross Beater Hammer Mill SK 1). Residue subsamples (five per crop) were analyzed for total N using the Kjeldahl method and for ammonium and nitrate (NH_4^+ and NO_3^-) contents by molecular absorption spectrophotometry in a segmented flow system (San Plus, Skalar, Breda, the Netherlands), after extraction with 1 M potassium chloride (KCl; 1:5 w/w). Total organic C was determined by combustion in a Primacs TOC Analyzer (Slakar Analytical B.V., Breda; the Netherlands). Soluble organic carbon and soluble organic N were determined by NIRD (near infrared detection) and chemiluminescence detection, respectively, in a Formacs analyzer (Skalar) after extraction with 0.01M calcium chloride ($CaCl_2$). Cellulose, hemicellulose and lignin contents were determined by proximate analysis (Goering & Van Soest, 1970).

Incubation experiment

As a representation of different temperature regimes and different residue managements under field conditions, two soil temperatures (10 and 20 °C) and two residue placements (on the surface and incorporated into the soil) were respectively designated for each of the three legume residues (cowpea, faba bean and pea). Thus, 12 treatments resulted from the combination of these three factors, plus two control treatments (soil without residues), one for each temperature.

According to the assigned placement treatment, residue particles were homogeneously incorporated into the pre-incubated soil or applied as an even single layer to its surface, at a rate of 12 g dry matter kg^{-1} dry soil (corresponding to 6 Mg dry matter ha^{-1}). This dosage was chosen to represent average residue yields in Europe for pea (Corre-Hellou & Crozat, 2005; Monti *et al*., 2016), faba bean (Jensen *et al*., 2010; Volpi *et al*., 2018) and cowpea (Kwapata & Hall, 1990; study in Mediterranean California, USA, as no information could be found for Europe). The soil (80 g dry weight) with and without residues was placed into cylindrical beakers (45 mm Ø × 80 mm length) and gently compacted to achieve the approximate bulk density of the original soil (1.20 g cm^{-3}). Soil moisture was corrected to 60% WFPS and kept constant by weighing the beakers every two or three days and adding the required amount of distilled water. Trays with water were kept inside the temperature-controlled chambers so as to reduce moisture loss from soil. The incubation experiment was carried out under aerobic conditions in the dark at the constant temperatures of 10 °C or 20 °C (according to designated treatment) for 240 days.

Soil was destructively sampled after 0, 3, 7, 14, 28, 56, 120 and 240 days: beakers were randomly withdrawn from the temperature-controlled chambers and immediately stored at -20 °C. After the end of the experiment, samples were slowly thawed at 4 °C and mineral N was immediately extracted with 1 M KCl (1:5 w/w) and analyzed for inorganic N using the previously described analytical procedure. Net N mineralized was calculated by the following equation:

$$\text{Net N mineralized} = (Ntreat_t - Ncontrol_t) - (Ntreat_0 - Ncontrol_0) \quad (1)$$

where $Ntreat_t$ is the soil mineral N content of the treated soil at day t, is the soil mineral N content of the respective control soil at day t, is the initial (day zero, immediately after adding residue) soil mineral N content of the treated soil, and is the initial soil mineral N content of the respective control soil at day zero. The following assumptions were made: priming effects were negligible, either because the addition of residues did not affect native soil organic N mineralization (no priming effect existed) or because each residue affected native soil organic N mineralization similarly (equivalent priming effects); gaseous losses of N were negligible (*i.e.* denitrification and volatilization were of a lower order of magnitude than soil N changes), since incubation was done in aerobic conditions (Recous *et al.*, 1995). Thus, positive and negative changes in net N mineralized in this study are exclusively attributed to mineralization and immobilization processes, respectively.

Statistical analysis and experimental design

ANOVA analysis of the residue chemical characteristics was performed in a completely randomized design with five replicates. ANOVA analysis of net N mineralized was performed in a completely randomized design with four replicates and four factors: residue, placement, temperature, and time. Simple effects analysis was used to assess the effect of each factor within all levels of the interacting factors. Differences between the means were separated by the Tukey's test at the probability level of 0.05. The linear correlation between residue characteristics and net N mineralized at each sampling day was determined by the Pearson's correlation coefficient and tested for significance at $\alpha = 0.05$.

Results

Legume residue characteristics

Overall, the three legume residues showed different chemical characteristics, but cowpea was the most distinctive residue. The highest total N content was found in this residue, 1.7 and 1.9 times higher than faba bean and pea, respectively (Table 1). Soluble organic N was also highest in cowpea, more than double than in faba bean or pea. Cowpea residue had substantial NH_4^+-N contents, contrary to faba bean and pea, both with negligible NH_4^+-N contents. All residues had neglectable NO_3^--N contents. Total and soluble C:N ratios were much lower in cowpea than in faba bean or pea. While soluble C:N ratios were comparable to total C:N ratios within faba bean and pea residues, cowpea soluble C:N ratio was less than half its total C:N ratio. Faba bean had the lowest cellulose content, significantly lower than both cowpea and faba bean, which were statistically similar. Cowpea had a higher hemicellulose content than the other two residues, which did not differ statistically. Lignin content was similar amongst residues (Table 1). The different N contents were thus the major contributor to differences in Lignin:N ratios among residues.

Total N added to soil by residues varied with legume species, due to differences in total N contents (Table 1). A total of 277, 165 and 149 mg Kjeldahl-N kg^{-1} soil (or 139, 83 and 75 kg Kjeldahl-N ha^{-1}) was added to the soil by cowpea, faba bean and pea residues, respectively, at the applied dose of 6 t dry residue ha^{-1}. Likewise, due to differences in mineral N ($NH_4^+ + NO_3^-$) contents among species (Table 1), inorganic N directly added to the soil by cowpea, faba bean and pea residues was 23, 4 and 5 mg N kg^{-1} soil (equivalent to 12, 2 and 3 kg mineral N ha^{-1}), respectively. This inorganic N was not considered as net N mineralized, since the mineral N content of the treated soil at day zero (immediately after residue addition) was subtracted from the mineral N content of the treated soil at each day (Equation 1).

Effect of legume species on net N mineralization

Net N mineralized was positively and significantly correlated with total N ($r = 0.58$), soluble N ($r = 0.58$), cellulose ($r = 0.46$) and hemicellulose ($r = 0.60$) contents (Table 2). On the contrary, C:N ($r = –0.56$) and Lignin:N ($r = –0.60$) ratios were negatively correlated with net N mineralized. No significant correlations were found between the net N mineralized and lignin content ($r = 0.00$; Table 2).

Net N mineralized evolved significantly with incubation time in all treatments, except in soil incubated at 10 °C with faba bean at both placements or with pea placed on the surface, where no significant net N mineralization occurred with time. Average

Table 1. Chemical characteristics of the legume residues ($n = 5$).

Residue	Total N	Sol N	CEL	HEM	LIG	NH_4^+-N	NO_3^--N	C:N	Sol (C:N)	LIG:N
	(g kg^{-1})						(mg kg^{-1})			
Cowpea	23.1a	6.7a	323.7a	127.1a	102.7	1.9a	14.7	16.6c	6.9b	4.4b
Faba bean	13.7b	3.0b	267.8b	102.1b	106.6	0.3b	24.6	29.9b	32.3a	7.8a
Pea	12.4c	2.5c	314.4a	110.6b	94.5	0.4b	36.9	33.0a	30.9a	7.6a
SEM	1.3	0.5	7.4	3.3	3.6	0.2	4.0	1.9	3.1	0.5

Sol: soluble. CEL: cellulose. HEM: Hemicellulose. LIG: lignin. Within each line, values followed by different letter are significantly different at $p < 0.05$.

Table 2. Linear correlation coefficients between net N mineralized and initial chemical characteristics of the legume residues (n = 4).

Time (days)	Total N	C:N	CEL	HEM	LIG	LIG:N	Sol N	Sol (C:N)
3	0.82***	–0.80***	0.64***	0.84***	0.02	–0.84***	0.82***	–0.84***
7	0.41**	–0.39**	0.47***	0.50***	–0.16	–0.45**	0.41**	–0.45**
14	0.28	–0.26	0.32*	0.34*	–0.11	–0.30*	0.28	–0.31*
28	0.49***	–0.48***	0.40**	0.51***	0.00	–0.51***	0.49***	–0.51***
56	0.61***	–0.60***	0.36*	0.57***	0.14	–0.61***	0.61***	–0.61***
120	0.73***	–0.72***	0.52***	0.72***	0.07	–0.74***	0.73***	–0.74***
240	0.71***	–0.70***	0.51***	0.70***	0.07	–0.72***	0.71***	–0.72***

CEL: cellulose. HEM: hemicellulose. LIG: lignin. Sol: soluble. *, **, ***: significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

changes in net N mineralized over all treatments were fast in the first 14 days (-653 µg N kg^{-1} soil day^{-1}) and slow from days 14 to 240 (82 µg N kg^{-1} soil day^{-1}). A three-way interaction was observed amongst residue, temperature and incubation time (Table 3). Net N mineralized was always significantly higher (with few occasional exceptions) in soil with cowpea residues than in soil with faba bean or pea residues incubated at the same temperature (Fig. 1). Net N mineralized never differed significantly between soil with faba bean and soil with pea, when incubated at similar temperatures for the same time period.

Soil with cowpea residues had a peak in net N mineralized (up to 23 mg N kg^{-1}) after three days, followed by a quick N immobilization until day 14 when incubated at 20 °C and until day 28 when incubated at 10 °C (Fig. 1). Finally, N mineralization occurred after this period until the end of the incubation, but net N immobilization was still observed until day 56 when cowpea was incubated at 10 °C. Net N mineralized at day 240 reached between 19 to 66 mg N kg^{-1} soil (incorporated at 10 °C and on the surface at 20 °C, respectively; data not shown), which corresponds to 7–24% of the N added by cowpea residues.

Table 3. Factorial analysis of variance (ANOVA) results (p values) for the net N mineralized by legume residues.

Factor	Significance
Residue	***
Placement	***
Temperature	ns
Time	***
Residue × Placement	*
Residue × Temperature	***
Residue × Time	***
Placement × Temperature	ns
Placement × Time	***
Temperature × Time	***
Residue × Placement × Temperature	ns
Residue × Placement × Time	ns
Residue × Temperature × Time	**
Placement × Temperature × Time	ns
Residue × Placement × Temperature × Time	ns

*, **, ***: significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; ns: not significant.

Patterns of N mineralization were similar between faba bean and pea residues, irrespective of incubation temperature (Fig. 1). Overall, they consisted of a rapid N immobilization in the first three days, followed by a slow N mineralization until day 7 or 14, then a gradual N immobilization until day 56 or 120 and finally a slow N mineralization until day 240. Net N immobilization was observed throughout the entire incubation in most treatments with faba bean or pea residues, and reached its peak at day 56 or 120 (Fig. 1), with up to 38 mg immobilized-N kg^{-1} soil (in soil with incorporated faba bean at 20 °C; data not shown). Soil mineral N reached low levels (< 10 mg N kg^{-1} soil) in all soil with incorporated faba bean and pea, at both temperatures, but complete depletion was never observed (minimum value was 7 mg N kg^{-1} soil; data not shown). After 240 days, net N mineralized in soil with faba bean or pea residues varied between –19 and 14 mg N kg^{-1} soil (respectively for faba bean incorporated at 20 °C and pea applied to surface at 20 °C; data not shown). Less than 10% of N added by these two legume residues was made available in the 240 days.

Effect of placement on N mineralization

A two-way interaction was observed between Placement and Residue, and Placement and Incubation time (Table 3). Soil with residues on the surface had significantly higher net N mineralized than soil with residues incorporated at all incubation times longer than seven days (Fig. 2). After the 240 days, positive net N mineralization was observed in all treatments with residues on the surface, except in faba bean at 10 °C (–2 mg N kg^{-1}; data not shown), with cowpea at 20 °C presenting the highest value (66 mg N kg^{-1}; data not shown). In treatments with incorporated residues, soil with cowpea presented positive net N mineralization after the 240 days (19 – 37 mg N kg^{-1};

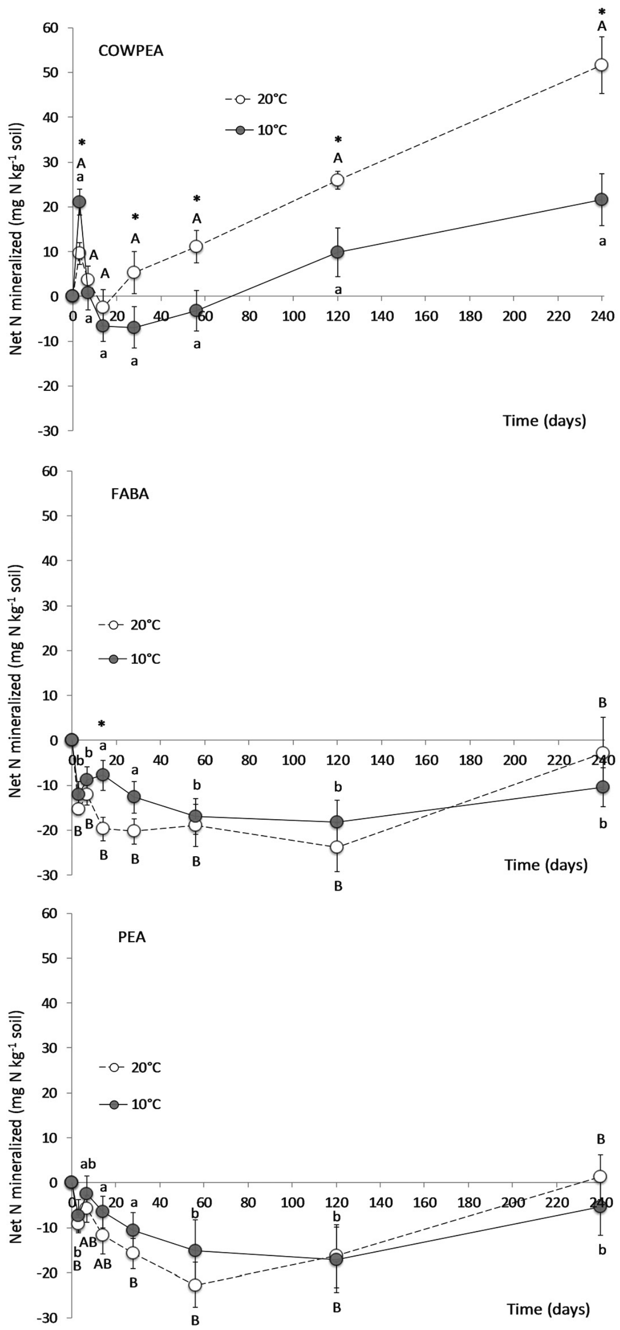

Figure 1. Net N mineralized in soil with cowpea, faba bean and pea residues, incubated at 10 °C and at 20 °C. Within each chart, significant differences between the two incubation temperatures at each sampling time are signaled with an asterisk. Within each temperature and incubation time, residues with different letters are statistically different at $p < 0.05$ (Tukey's test): lowercase letters for 10 °C and uppercase letters for 20 °C. Vertical bars represent the standard error of the mean. Where bars are not visible, symbols are larger than errors.

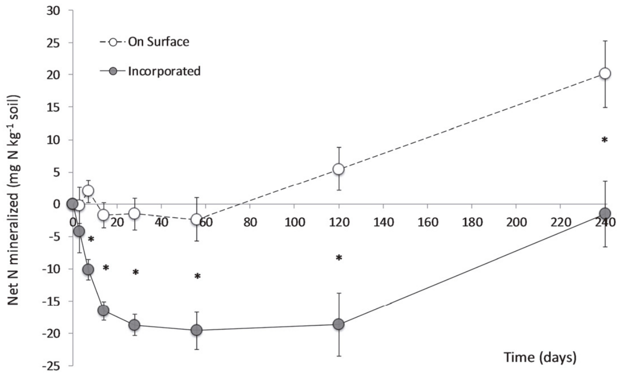

Figure 2. Net N mineralized in soil with legume residues incorporated and applied to the surface. Significant differences between the two placements at each sampling time are signaled with an asterisk. Vertical bars represent the standard error of the mean.

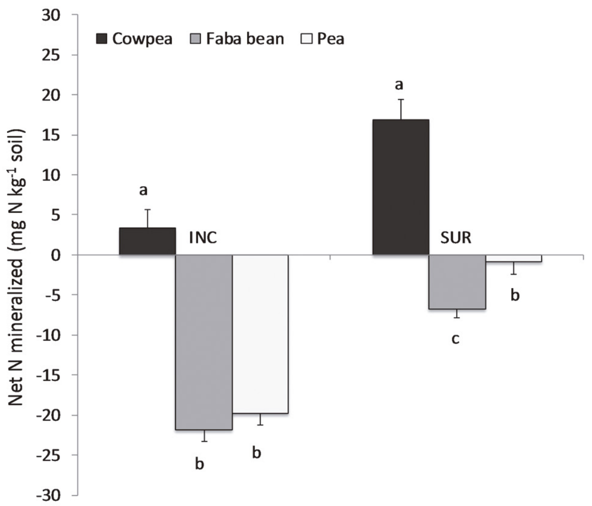

Figure 3. Average net N mineralized in soil with cowpea, faba bean and pea residues, incorporated (INC) and applied to the soil surface (SUR) for all incubation times and temperatures. Within each placement, residues with different letters are statistically different at $p < 0.05$ (Tukey's test). Vertical bars represent the standard error of the mean.

data not shown), while negative net N mineralization was observed in all soil with faba bean or pea residues (between –19 and –12 mg N kg^{-1}; data not shown).

Average net N mineralized was significantly higher in soil with cowpea than in soil with faba bean or pea at both placements (Fig. 3). Average net N mineralized in soil with pea was higher than in soil with faba bean, but differences were only significant when residues were left on the soil surface. Differences in net N mineralized between the two placements increased with increasing initial C:N ratios of the residues (13.5, 15.0 and 19.0 mg N kg^{-1} on average for all days for cowpea, faba bean and pea, respectively; Fig. 3).

Effect of temperature on N mineralization

Temperature did not significantly affect net N mineralized in soil with faba bean or pea residues, regardless of incubation time (with the single exception of day 14 in soil with faba bean residues). On the contrary, temperature significantly affected net N mineralized in soils with cowpea residues at most incubation days, with days seven and 14 as the only exceptions (Fig. 1).

Discussion

Legume residue characteristics

The C:N ratios of cowpea residue (Table 1) were below the usually reported thresholds between net N mineralization and N immobilization, anticipating a typical mineralization process, with no N immobilization throughout the entire incubation period. On the contrary, the relatively high C:N ratios of faba bean and pea residues (Table 1) anticipated an immobilization-mineralization process (Trinsoutrot *et al.*, 2000; Chen *et al.*, 2014). The higher quality of the cowpea residues may be attributed to more than one factor. First of all, cowpea residues seem to have an intrinsically lower C:N ratio (21, Nishigaki *et al.*, 2017; 24-29, Franzluebbers *et al.*, 1994) than those of faba bean (27, Jensen *et al.*, 2010; 39, Badagliacca *et al.*, 2017) and pea (38-56, Engström & Lindén, 2012; 28, Kumar & Goh, 2002). However, the C:N ratio of cowpea in the present study (17; Table 1) was below the range reported for mature residues of this species. The use of irrigation during cultivation may explain this result, as it prevented terminal drought stress, which is known to decrease the total and soluble organic N contents and increase the soluble and total C:N ratios (Chen *et al.*, 2015). On the contrary, the C:N ratio of faba bean and pea residues (Table 1) were within the reported ranges. These residues were obtained from rainfed crops subjected to terminal drought stress (data not shown), which is typical in winter crops under Mediterranean conditions (Daryanto *et al.*, 2015). Thus, differences in residue quality between legume crops seem to have resulted from a combination of intrinsic characteristics of each species and agricultural management practices.

Effect of legume species on N mineralization

As anticipated by the differences in C:N ratios, net N mineralization patterns of cowpea were clearly distinct from those of faba bean and pea (Fig. 1). The net N mineralization flush observed in the first three days in soil with cowpea residue (Fig. 1) can be justified by the low soluble C:N ratio of this residue (Table 1), as the soluble components are labile and rapidly decomposed (Hadas *et al.*, 2004). Correspondingly, the quick N immobilization immediately after faba bean and pea residue addition to soil (Fig. 1) can be attributed to their relatively high soluble C:N ratios (Table 1). The strong negative correlation between the soluble C:N ratios and the net N mineralized in the first three days (Table 2) support this theory. On the contrary to what was anticipated in soils with cowpea residue, a period of N immobilization followed the initial flush of N (Fig. 1). This was likely due to depletion of the N-rich soluble compounds and to increased microbial N requirements for decomposition of the non-soluble compounds, due to their higher C:N ratio (20.6; Table 1) than the C:N ratio of soluble compounds (6.7; Table 1). However, the 23 mg mineral N kg^{-1} soil added directly by cowpea residues (not considered as net N mineralized), can counterbalance the negative net N mineralized values observed in soil with incorporated cowpea (as low as -17 mg N kg^{-1} soil after 28 days at 10 °C; data not shown), meaning that no true negative N changes occurred in these treatments compared with the control soil.

The overall decreased rates of changes in net N mineralized after the initial 14 days indicate the onset of decomposition of cellulose and hemicellulose (Recous *et al.*, 1995). The low levels of soil inorganic N, particularly when residues of the winter-grown legumes were incorporated into the soil (< 10 mg N kg^{-1} soil; data not shown), also likely slowed decomposition due to limited microbial activity (Recous *et al.*, 1995). This can explain why net N mineralized in these treatments was still negative at the end of the trial, despite its considerable time-span (between –19 and –12 mg N kg^{-1}; data not shown). Lignin content has also often been negatively correlated with nitrogen mineralization at the later stages of decomposition (Corbeels *et al.*, 2003; Vahdat *et al.*, 2011). This effect has been attributed to its recalcitrance and resistance to microbial degradation (Frei, 2013) and to protection of other cell-wall constituents from decomposition due to limited microbial enzyme accessibility (Pauly & Keegstra, 2008; Bhatnagar *et al.*, 2018). However, when low, lignin content is not generally regarded as a good predictor of net N mineralization (Trinsoutrot *et al.*, 2000; Corbeels *et al.*, 2003). The low lignin content of the studied residues (Table 1) and the lack of a significant linear correlation between lignin content and net N mineralized (Table 2) support this premise. However, the low variability in lignin contents within the three legume residues (Table 1) could also explain the absence of a correlation. Still, cellulose and hemicellulose contents were always significantly and positively correlated with net N mineralized (Table 2), indicating that lignin encapsulation did not substantially impair the degradation of these compounds. The low initial soil pH (4.9) is not likely to have affected mineralization overall, as ammonification is usually not constrained by acidity at pH above 4 (Xiao *et al.*, 2013; Wang *et al.*, 2017).

In all, taking into consideration the net N mineralized after 240 days and the inorganic N added directly by residues (as discussed above), cowpea residues released an equivalent of 21–45 kg mineral N ha^{-1} to soil, a considerable contribution to soil N-fertility. Previous studies in field conditions have shown comparable increases in N uptake by cereals grown in soil with incorporated cowpea residue. Nishigaki *et al.* (2017) reported that maize N uptake increased 9.6–23.8 kg N ha^{-1} with the incorporation of 2.7 Mg ha^{-1} cowpea residue, as compared with no residue addition, in two different soils in Tanzania. Incorporating 2 Mg ha^{-1} cowpea residue into the soil also increased the N uptake of subsequent pearl millet by 13-24 kg N ha^{-1} when compared to soil with no residue in a sandy soil in Niger (Franzluebbers *et al.*, 1994). On the contrary, faba bean and pea residues seem able to release at best 10 kg mineral N ha^{-1} in the time period of this incubation, and can immobilize up to 17 kg mineral N ha^{-1} during decomposition. Similar findings were reported from a field incubation study in Sweden, where the net N immobilization after incorporating pea residues (9 kg N ha^{-1}) was similar to that observed in the present trial (5-10 kg N ha^{-1}), in the same time-frame (Engström & Lindén, 2012). To our knowledge, no studies reporting the net N mineralized from mature faba bean residues had been published to date.

Despite the good correspondence between results obtained in different experiments, it should be noted that some limitations exist in the translation of observed net N changes under laboratory to field conditions. First of all, ground residues were used in the incubation, as compared to the usually chopped residues in the field. This reduced particle size is known to increase availability and accessibility of N to microbes and could have thus artificially increased decomposition rates (Corbeels *et al.*, 2003). Mesofauna is also known to affect residue decomposition, particularly in soils under intensive agriculture (Castro-Huerta *et al.*, 2015), but was absent in the laboratory.

Effect of placement on N mineralization

As anticipated, less soil inorganic N immobilization occurred when residues were left on the soil surface rather than incorporated into the soil (Fig. 2), in good agreement with analogous studies with various types of soil (Coppens *et al.*, 2006; Abiven & Recous, 2007; Li *et al.*, 2013). This reduction in N mineralization/immobilization rates at the surface has been attributed to slower decomposition rates resulting from the decreased contact area between residues and soil microorganisms (Coppens *et al.*, 2006).

The effect of placement in N mineralization was more pronounced in faba bean and pea than in cowpea residues (Fig. 3). Kumar & Goh (2002) also found larger differences in the N mineralized between incorporated and surface-applied residues when their C:N ratios were higher. They justified this result with a greater N immobilization when high C:N residues were incorporated rather than left on the soil surface. In the present study, N was likely a limiting factor to decomposition when faba bean and pea (but not cowpea) residues were incorporated into the soil (as discussed above). The resulting slower remineralization of the immobilized N can explain the long period of net N immobilization that occurred in these treatments and the resulting negative net N mineralization after the 240 days (between –19 and –12 mg N kg^{-1} soil; data not shown), which may have also contributed to the greater effect of placement in these legumes as compared with cowpea (Fig. 3).

These results suggest that all three legume residues release N faster to the soil under no-tillage rather than conventional tillage, but also that cowpea residues should be less affected by tillage system. The effect of tillage on mineralization of native soil organic matter, which can be relevant due to the disruption of soil aggregates, increased aeration and decreased moisture content, was however not regarded in the present study (Balesdent *et al.*, 2000). Moisture limitation has been reported as more important than N limitation in the decomposition of residues at the soil surface (Coppens *et al.*, 2006). However, as soil moisture was kept constant throughout the incubation, this effect was also not taken into account. Other limitations can be appointed to the translation of residue placement effects in this trial to field conditions. Daily fluctuations of temperature can affect N mineralization (Sierra, 2002). In field conditions, residues left on the surface will sustain higher amplitude of temperature variations than incorporated residues (Georgopoulos & Bartzokas, 2018), which was not represented in the present study. Additionally, although lignin decomposition in soil is predominantly carried out by brown rot and white rot fungi and some species of bacteria, photo-degradation can also occur (Frei, 2013). For this reason, a higher rate of lignin degradation can be expected when residues are placed on the surface, due to exposure to sunlight. However, our study was conducted in the dark, so this effect was also overlooked.

Effect of temperature on N mineralization

The lower effect of incubation temperature on the mineralization of faba bean and pea may be partly explained by the overall lower rates of N mineralization of these residues when compared with those of cowpea (Fig. 1). Changes in decomposer microflora communities could also help explain these observations. Giacomini *et al*. (2007) suggested that the decomposer microflora adapts to low soil N content through an increased proportion of fungi to bacteria, due to the lower N requirements of fungi. Hence, decomposition of faba bean and pea was possibly more fungal-driven than in cowpea, due to the low N levels in soil with the former residues (as discussed above). Fungi are better adapted to cold conditions than bacteria, due to lower optimum and maximum temperatures for growth and activity (Siles *et al.*, 2019). Siles *et al.* (2019) reported that bacterial biomass was significantly decreased at 10 °C as compared to 20 °C in a soil incubation with added cellulose (which can be decomposed by both fungi and bacteria; Bhatnagar *et al.*, 2018; Siles *et al.*, 2019), while fungal biomass remained unchanged. This indicates a lower effect of temperature on residue mineralization when fungi dominate the decomposition process, which could justify the lower sensitivity of faba bean and pea N mineralization to changes in temperature when compared with cowpea. However, further studies including data on microbial communities are required to confirm this postulation.

These results indicate that different temperature regimes should not significantly affect net N mineralization of faba bean and pea residues. On the contrary, the potential mineral N release from cowpea residues seems to increase in warmer climates. It should however be noted that, as previously discussed, the effect of diurnal temperature fluctuations in the net N mineralized under field conditions was not represented in this trial, as temperatures were kept constant. The differences in moisture regimes between distinct climates were not represented in the trial, but may also drastically change mineralization patterns.

In summary, out of the three legume residues studied, cowpea showed the highest quality and potential for improving soil mineral N availability, as no negative N changes occurred and between 21-45 kg N ha^{-1} (at typical legume residue yields) were released in the eight months of the soil incubation. On the contrary, faba bean and pea residues immobilized N throughout most of this time period (as much as 17 kg N ha^{-1} soil). These results suggest that application of cowpea residues to soil is more appropriate when high N levels are required shortly after, for example when followed by a crop with high initial N requirements. On the other hand, application of faba bean and pea residues seems more appropriate when aiming for the conservation of soil N for later release, such as when a fallow season succeeds.

As in most previous studies, higher net N mineralization occurred when legume residues were applied to the soil surface rather than incorporated, indicating that surface-application of these residues could be advantageous if a subsequent crop is sown shortly after residue addition. However, if a fallow season ensues after the addition of residues with low C:N ratio, incorporating the residues should be a suitable strategy to mitigate N losses.

Cowpea residues seem able to release significantly more mineral N to soil when decomposing in warmer climates, while N mineralization from faba bean and pea should be less affected by temperature. Sensitivity of net N mineralization to temperature decreased with increasing residue C:N ratios, which was attributed to lower rates of mineralization and to possible changes in decomposer microflora composition and associated N and temperature requirements for optimum activity.

These findings contribute to a better understanding of observations from studies under field conditions, where soil N dynamics depends on many factors. Studies on the response of decomposer microflora communities to different crop residues and managements are desirable for a better understanding of residue mineralization processes.

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